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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12Q 1/68	A2	(11) International Publication Number: WO 00/50639 (43) International Publication Date: 31 August 2000 (31.08.00)
(21) International Application Number: PCT/US00/01392 (22) International Filing Date: 20 January 2000 (20.01.00) (30) Priority Data: 60/121,047 22 February 1999 (22.02.99) US 60/139,440 15 June 1999 (15.06.99) US 09/357,743 20 July 1999 (20.07.99) US (71) Applicant (for all designated States except US): VARIAGEN-ICS, INC. [US/US]; 60 Hampshire Street, Cambridge, MA 02139-1562 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): STANTON, Vincent, Jr. [US/US]; 32 Royal Road, Belmont, MA 02173 (US). (74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon LLP, 633 West Fifth Street, Suite 4700, Los Angeles, CA 90071-2066 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: GENE SEQUENCE VARIATIONS WITH UTILITY IN DETERMINING THE TREATMENT OF DISEASE		
(57) Abstract The present disclosure describes the use of genetic variance information for genes involved in gene pathways in the selection of effective methods of treatment of a disease or condition. The variance information is indicative of the expected response of a patient to a method of treatment. Methods of determining relevant variance information and additional methods of using such variance information are also described.		

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DESCRIPTION

GENE SEQUENCE VARIATIONS WITH UTILITY IN DETERMINING THE TREATMENT OF DISEASE

BACKGROUND OF THE INVENTION

This application concerns the field of mammalian therapeutics and the selection of therapeutic regimens utilizing host genetic information, including gene sequence variances within the human genome in human populations.

The information provided below is not admitted to be prior art to the present invention, but is provided solely to assist the understanding of the reader.

Many drugs or other treatments are known to have highly variable safety and efficacy in different individuals. A consequence of such variability is that a given drug or other treatment may be effective in one individual, and ineffective or not well-tolerated in another individual. Thus, administration of such a drug to an individual in whom the drug would be ineffective would result in wasted cost and time during which the patient's condition may significantly worsen. Also, administration of a drug to an individual in whom the drug would not be tolerated could result in a direct worsening of the patient's condition and could even result in the patient's death.

For some drugs, over 90% of the measurable variation in selected pharmacokinetic parameters has been shown to be heritable. For a limited number of drugs, geneDNA sequence variances have been identified in specific genes that are involved in drug action or metabolism, and these variances have been shown to account for the variable efficacy or safety of the drugs in different individuals. As the sequence of the human genome is completed, and as additional human gene sequence variances are identified, the power of genetic methods for predicting drug response will further increase.

In this invention, we address the difficulties that arise in treating the following disease categories: 1) neurological and psychiatric disease; 2) pharmacokinetic and dynamic indices including efficacy, absorption, distribution, metabolism, and excretion, as well as safety and toxicity parameters; 3) inflammation and immune disease; 4) endocrine and metabolic disease; 5) cardiovascular and renal disease; and 6) cancer.

Neurological and Psychiatric Disease

Diseases of the central nervous system (CNS) present unique medical challenges to clinicians, patients, and caregivers. These diseases often progress to severely debilitating conditions. Further, the efficacy of available treatments is limited and there are serious, mostly unpredictable, side effects associated with some drugs. The progressive nature of neurological and psychiatric disease makes the passage of time a crucial issue in the treatment process. Specifically, selection of optimal treatment for neurological and psychiatric diseases is complicated by the fact that it often takes weeks or months to determine if a given therapy is symptomologyproducing a measurable benefit. Thus the current empirical approach to prescribing pharmacotherapy, in which each course of treatment for a given patient is a small experiment, is unsatisfactory from both a medical and economic perspective. Even when an effective treatment is ultimately identified, it often follows a period of ineffective or suboptimal treatment.

Pharmacokinetic and Pharmacodynamic Effects

The efficacy of a drug is a function of both pharmacodynamic effects and pharmacokinetic effects, or bioavailability. In the present invention, interpatient variability in drug safety, tolerability and efficacy are discussed in terms of the genetic determinants of interpatient variation in absorption, distribution, metabolism, and excretion, i.e. pharmacokinetic parameters.

Adverse drug reactions are a principal cause of the low success rate of drug development programs (less than one in four compounds that enters human clinical testing is ultimately approved for use by the US Food and Drug Administration (FDA)). Adverse drug reactions can be categorized as 1) mechanism based reactions and 2) idiosyncratic, "unpredictable" effects apparently unrelated to the primary pharmacologic action of the compound. Although some side effects appear shortly after administration, in some instances side effects appear only after a latent period. Adverse drug reactions can also be categorized into reversible and irreversible effects. The methods of this invention are useful for identifying the genetic basis of both mechanism based and 'idiosyncratic' toxic effects, whether reversible or not. Methods for identifying the genetic sources of interpatient variation in efficacy and mechanism based toxicity may be initially directed to analysis of genes affecting pharmacokinetic parameters, while the genetic causes of idiosyncratic adverse drug reactions are more likely to be attributable to genes affecting variation in pharmacodynamic responses or immunological responsiveness.

Absorption is the first pharmacokinetic parameter to consider when determining the causes of intersubject variation in drug response. The relevant genes depend on the route of administration of the compound being evaluated. For

orally administered drugs the major steps in absorption may occur during exposure to salivary enzymes in the mouth, exposure to the acidic environment of the stomach, exposure to pancreatic digestive enzymes and bile in the small intestine, exposure to enteric bacteria and exposure to cell surface proteins throughout the gastrointestinal tract. For example, uptake of a drug that is absorbed across the gastrointestinal tract by facilitated transport may vary on account of allelic variation in the gene encoding the transporter protein. Many drugs are lipophilic (a property which promotes passive movement across biological membranes). Variation in levels of such drugs may depend, for example, on the enterohepatic circulation of the drug, which may be affected by genetic variation in liver canalicular transporters, or intestinal transporters; alternatively renal reabsorption mechanisms may vary among patients as a consequence of gene sequence variances. If a compound is delivered parenterally then absorption is not an issue, however transcutaneous administration of a compound may be subject to genetically determined variation in skin absorptive properties.

Once a drug or candidate therapeutic intervention is absorbed, injected or otherwise enters the bloodstream it is distributed to various biological compartments via the blood. The drug may exist free in the blood, or, more commonly, may be bound with varying degrees of affinity to plasma proteins. One classic source of interpatient variation in drug response is attributable to amino acid polymorphisms in serum albumin, which affect the binding affinity of drugs such as warfarin. Consequent interpatient variation in levels of free warfarin have a significant effect on the degree of anticoagulation. From the blood a compound diffuses into and is retained in interstitial and cellular fluids of different organs to different degrees. Interpatient variation in the levels of a drug in different anatomical compartments may be attributable to variation in the genetically encoded chemical environment of those tissues (cell surface proteins, matrix proteins, cytoplasmic proteins and other factors)

Once absorbed by the gastrointestinal tract, compounds encounter detoxifying and metabolizing enzymes in the tissues of the gastrointestinal system. Many of these enzymes are known to be polymorphic in man and account for well studied variation in pharmacokinetic parameters of many drugs. Subsequently compounds enter the hepatic portal circulation in a process commonly known as first pass. The compounds then encounter a vast array of xenobiotic detoxifying mechanisms in the liver, including enzymes which are expressed solely or at high levels only in liver. These enzymes include the cytochrome P450s, glucuronyltransferases, sulfotransferases, acetyltransferases, methyltransferases, the glutathione conjugating system, flavine monooxygenases, and other enzymes known

in the art. Polymorphisms have been detected in all of these metabolizing systems, however the genetic factors responsible for intersubject variation have only been partially identified, and in some cases not yet identified at all. Biotransformation reactions in the liver often have the effect of converting lipophilic compounds into hydrophilic molecules which are then more readily excreted. Variation in these conjugation reactions may affect half-life and other pharmacokinetic parameters. It is important to note that metabolic transformation of a compound not infrequently gives rise to a second or additional compounds that have biological activity greater than, less than, or different from that of the parent compound. Metabolic transformation may also be responsible for producing toxic metabolites.

Biotransformation reactions can be divided into two phases. Phase I are oxidation-reduction reactions and phase II are conjugation reactions. The enzymes involved in both of these phases are located predominantly in the liver, however biotransformation can also occur in the kidney, gastrointestinal tract, skin, lung and other organs. Phase I reactions occur predominantly in the endoplasmic reticulum, while phase II reactions occur predominantly in the cytosol. Both types of reactions can occur in the mitochondria, nuclear envelope, or plasma membrane. One skilled in the art can, for some compounds, make reasonable predictions concerning likely metabolic systems given the structure of the compound. Experimental means of assessing relevant biotransformation systems are also described.

Drug-Induced Disease, Disorders or Toxicities

Drug-induced disease or toxicity presents a unique series of challenges to drug developers, as these reactions are often not predictable from preclinical studies and may not be detected in early clinical trials involving small numbers of subjects. When such effects are detected in later stages of clinical development they often result in termination of a drug development program because, until now, there have been no effective tools to seek the determinants of such reactions. When a drug is approved despite some toxicity its clinical use is frequently severely constrained by the possible occurrence of adverse reactions in even a small group of patients. The likelihood of such a compound becoming first line therapy is small (unless there are no competing products). Thus clinical trials that lead to detection of genetic causes of adverse events and subsequently to the creation of genetic tests to identify and screen out patients susceptible to such events have the potential to (i) enable approval of compounds for genetically circumscribed populations or (ii) enable repositioning of approved compounds for broader clinical use.

Similarly, many compounds are not approved due to unimpressive efficacy. The identification of genetic determinants of pharmacokinetic variation may lead to identification of a genetically defined population in whom a significant response is

occurring. Approval of a compound for this population, defined by a genetic diagnostic test, may be the only means of getting regulatory approval for a drug. As healthcare becomes increasingly costly, the ability to allocate healthcare resources effectively becomes increasingly urgent. The use of genetic tests to develop and rationally administer medicines represents a powerful tool for accomplishing more cost effective medical care.

Inflammation and Immune Disease

In this application, we further address the difficulties that arise in treating inflammatory diseases and other diseases in which modulation of immunologic function provides the basis for therapeutic intervention, including, for example, diseases treated with antiinflammatory, analgesic or antipyretic drugs as well as autacoids, eicosanoids, interleukins, cytokines or their agonists or antagonists. Diseases or conditions involving the inflammatory response or immune system constitute a complex and heterogeneous group of diseases, involving all organ systems from the central nervous system and the circulatory system to the viscera and skin. The diseases may be acute or chronic, or may have an acute stage which later progresses to a chronic condition, or may exhibit a waxing and waning pattern of flare ups and remissions. Due to their wide anatomical distribution, this group of diseases can (collectively) lead to impairment of a wide range of essential physiological functions. The unifying theme in the treatment of these diseases is the modulation of inflammatory mediators or immune function. The evaluation of long term response to therapy is, for many of these diseases, the most important index of treatment efficacy, due to the progressive nature of inflammatory or immunological diseases. Since it is often difficult to assess the long term effects of treatment over a short observation period (particularly for diseases with a waxing and waning pattern) there is considerable utility in a genetic test that can predict long term outcomes. Many treatments for diseases with significant inflammatory or immunological components are quite costly.

Endocrine and Metabolic Disease

The endocrine system encompasses a number of organs that collectively regulate a wide array of physiologic, metabolic and developmental processes including metabolism, growth, reproduction, development, senescence, behavior, including adaptation to stress, the composition of intracellular and extracellular fluids (e.g. salt and water balance), digestion and wound healing, among other processes. The endocrine organs include the hypothalamus, pituitary gland, thyroid, parathyroid, endocrine pancreas, adrenal gland, gonads, and cells of the gastrointestinal tract, liver, kidneys, heart, pineal gland, and placenta.

Endocrine signals can be classified as autocrine, paracrine, or endocrine depending on the distance over which a signal must be transmitted. Endocrine signals are transmitted by hormones including peptides, proteins, steroids and small molecule neurotransmitters. The hormones carry biological signals to target cells.

5 Receptors located on the cell surface (membrane bound) activate intracellular second messenger systems to ultimately alter intracellular metabolism, physiology and cell function. Second messengers systems include adenylate cyclase, guanylate cyclase, phospholipases, and kinases. Some membrane receptors interact with GTP-binding proteins; others produce intracellular signals themselves (for example
10 receptors with tyrosine kinase domains). Other receptors are located intracellularly (for example steroid hormone receptors) and the hormone-receptor complex acts to stimulate intracellular processes such as gene transcription.

Regulation in the endocrine system occurs via a complex system of signals transmitted by hormones, neurotransmitters and other small molecules. These
15 signals participate in feedback loops, recruitment of coordinate responses, and cycles or rhythms. The feedback loops function to coordinately stimulate or terminate hormone signals. In this way, communication occurs between cells or
tissues that are physically separated. For example, a peripheral endocrine gland may release hormones in response to centrally produced stimulatory hormones, with the
20 peripherally produced substances feeding back on the central nervous system to decrease production of the stimulatory signal. In other systems the action of multiple hormones must be coordinated. For example, female reproductive system requires hypothalamic, pituitary and ovarian signals and also includes effector
targets in the breasts, uterus, and vagina. Endocrine signalling systems that are
25 regulated in a coordinated fashion include, for example, the hypothalamic-pituitary-gonadal axis, the hypothalamic-pituitary-adrenal corticotroph axis and the hypothalamic-pituitary-thyroid axis. Within the endocrine system there is integration of endocrine responses that are grouped as.

Many hormones are extensively processed prior to secretion. For example in
30 the posterior pituitary gland, a hormone gene encodes a preprohormone that contains several proteins or peptides in contiguous alignment that requires modification prior to becoming an active signaling hormone. The preprohormone after nascent ribosome synthesis then is cut by specific or nonspecific processing proteins to a smaller prohormone within the Golgi apparatus, that then is glycosylated and placed
35 into a secretory granule. Within the secretory granule, the prohormone is then further processed into the active hormone. The active hormone is secreted as a response to physiologic signals and renders the specific biologic function at the target organ or tissue. In this complex protein processing mechanism, there is the

possibility of secreting more than one hormones or signaling peptides in the same secretory granule, and as described above, can lead to the delivery of multiple signals to one or more target tissues.

Assessment of endocrine function can be conducted by quantitation of circulating hormones and metabolic products, stimulation and suppression tests, and anatomic assessment. Aberrations of endocrine disease, disorder, or dysfunction manifests clinically as either a deficiency or an excess of 1) endocrine function or 2) hormone production, or may be the result of loss of 1) feedback loops, 2) recruitment of hormone signals, or 3) cycles or pulsatile hormone secretion. Lastly, there may be genetic determinants of endocrine disease, for example mutations or polymorphisms in biosynthetic enzymes, hormone receptors, peptide hormone or small molecules, immune surveillance, tumor suppressor genes, and others such that these changes or differences from normally occurring proteins or molecules alters their functional pattern and the clinical manifestation is then characteristic of endocrine disease.

Endocrine or metabolic disease provide a unique series of complications for clinicians, patients, and care givers; the diseases often progress rapidly and disrupts a vast number of major life functions. The progressive nature of these disease syndromes makes the passage of time a crucial issue in the treatment process.

Treatment choices for endocrine or metabolic disorders and their associated pathologies, particularly those affecting major organs, e.g. coronary, hepatic, renal systems, are often complicated by the fact that it often takes a significant period of treatment to determine if a given therapy is effective. Accordingly, treatment with the most effective drug or drugs is often delayed while the disease continues to progress. A method that would allow one to predict which patients will respond to a specific therapy would provide physical and psychological benefits. As healthcare becomes increasingly inaccessible, the ability to allocate healthcare resources effectively also becomes more important.

Cardiovascular and Renal Disease

In this application, we address the difficulties that arise in treating cardiovascular and renal diseases, describe methods to enable more effective use of available therapeutics, and methods for developing new therapies. Diseases of the cardiovascular and renal systems often progress, over periods of years to decades, to severely debilitating and life threatening conditions. The efficacy of available treatments is limited and there are side effects associated with many of the drugs used to treat these diseases. Due to the progressive nature of many cardiovascular and renal diseases it is of great importance to select an effective therapeutic regimen at the time of diagnosis. The effectiveness of therapy is often

assessed by short-term measurements of surrogate markers (e.g. blood pressure, blood lipid levels or blood clotting parameters), however the important endpoints (e.g. myocardial infarction, thromboembolism, renal failure) occur (or are prevented) over the long term. Thus, the tools for selecting optimal therapy for individual patients are currently limited, and as a result some patients receive treatment from which they do not benefit, while other patients may not receive treatment that would produce significant benefit. The current empirical approach to prescribing pharmacotherapy, in which each course of treatment for a given patient is a small experiment (e.g. the selection of effective therapy for blood pressure control), is unsatisfactory from both a medical and economic perspective. Even when an effective treatment is ultimately identified, it often follows a period of ineffective or suboptimal treatment. Methods that would help caregivers predict which patients will exhibit beneficial therapeutic responses to which medications would provide both medical and economic benefits. As healthcare becomes increasingly costly, the ability to rationally allocate healthcare expenditures, and in particular pharmacy resources, becomes increasingly important. The methods of this invention provide a basis for selecting more efficacious pharmacotherapy of cardiovascular and renal diseases.

Neoplastic Disorders

In this application, we also address the difficulties that arise in treating neoplastic disease. Due to the often rapid progression and life-threatening nature of neoplastic diseases, both early detection and effective treatment are essential. Clearly, there would be great benefit to patients if therapies that will ultimately prove to be ineffective in curbing the progression of disease could be avoided initially, given the cost and often noxious side effects associated with such therapies. Many current therapies for neoplastic disease are targeted against processes such as cell growth and division that occur in both normal and cancerous tissues (albeit at different rates), resulting in pronounced toxicity to normal tissues. Toxic reactions are the most severe in tissues which proliferate rapidly, such as gastrointestinal epithelium and hematopoietic tissues, however serious adverse reactions also occur in other organs occasionally, including heart, kidney, liver, lung and brain. As a consequence of the narrow therapeutic index associated with most antineoplastic treatments, skillful choice of treatments (including the agents used and the dose, if the treatment involves drugs) must be made by the attending physician based not only upon the type of cancer and stage of dissemination, but on a number of additional factors including status of the patient's hematopoietic and myelogenic tissues, hepatic and renal function and age. Knowledge of genetic factors which would impact the choice of treatment based either on optimizing efficacy or

minimizing toxicity would greatly benefit cancer patients, because the efficacy of available treatments is limited and there are serious, mostly unpredictable, side effects associated with some drugs. Thus a method that would allow one to predict which patients will exhibit beneficial therapeutic response to a specific medication with minimal adverse effects (often less than half of treated patients, and not infrequently one quarter or less) would provide physical, psychological, and societal benefits. Using such a method, those patients not likely to benefit from aggressive treatment could be offered palliative care. Tumor growth exhibits gompertzian kinetics—growth rate declines with increasing tumor burden. Since chemotherapies are frequently most effective against rapidly growing tumors (low tumor burden), it is imperative that treatment begin immediately after disease detection and that the tumor responds to first-line therapy. Further, selection of optimal treatment for a neoplastic disease is complicated by the fact that it often takes weeks or months to determine if a given therapy is producing a measurable benefit. Thus the current empirical approach to prescribing pharmacotherapy, in which each course of treatment for a given patient is a small experiment, is unsatisfactory from both a medical and economic perspective. Even when an effective treatment is ultimately identified, it often follows a period of ineffective or suboptimal treatment.

Neoplastic diseases are related by the fact that they result from the unchecked growth of a previously normal cell, generally thought to be precipitated by one or more mutations in its genetic material. Cancerous cells can undergo gene loss and duplication to become aneuploid or partially polyploid, but usually retain some of the characteristics of their source tissue. Neoplastic cells differ in their ability to form solid tumors, to disseminate from the original site of tumor formation and form metastases, and in their requirements for growth factors, which can include steroid hormones in the case of carcinomas of the prostate or breast. Tumor cells, while having sustained alterations to their genetic material that lead either to a loss of growth inhibition or to a gain of growth function, still produce all the enzymes and other macromolecules required for cell viability. In this regard, they are extremely similar to non-cancerous tissue, and selective poisoning of tumor tissue over normal tissue has for the most part proven elusive. Current chemotherapies mainly target normal cell functions including DNA replication, cell division, RNA transcription, and nucleotide metabolism and are often associated with nausea and vomiting, diarrhea, hair loss, anemia, immune suppression (and consequent increased risk of infection), as well as a host of less common side effects including pulmonary fibrosis, and cardiac, hepatic and renal toxicity. Radiation therapy, often used in the treatment of inoperable tumors such as various brain and laryngeal tumors (but also widely used to treat breast cancer in patients who have had

lumpectomies), has the advantage that it can be restricted to a small area, especially when used in conjunction with tissue selective radiosensitizers or radioprotectants. Radiation therapy also targets rapidly proliferating tissues and shares many of the side effects of cytotoxic agents. Minimization of severe toxic reactions to cancer therapy through knowledge of genetic variances in normal tissue that could impact either drug metabolism or cellular repair processes would be an invaluable addition to cancer therapy.

Accordingly, a method that would help caregivers predict which patients will exhibit beneficial therapeutic responses to a specific which medication or medications would provide both medical and economic benefits. As healthcare becomes increasingly costly, the ability to rationally allocate healthcare expenditures, and in particular pharmacy resources, also becomes increasingly important.

SUMMARY OF THE INVENTION

The present invention is concerned generally with the field of identifying an appropriate treatment regimen for a neurological or psychiatric disease, drug-induced disease or disorders, endocrine or metabolic disease, inflammatory disease (or a disease in which modulation of the inflammatory response or the immune system is being tested for therapeutic effect), and cardiovascular and renal diseases, based upon genotype in mammals, particularly in humans. The present invention is additionally concerned generally with the field of pharmacology, specifically pharmacokinetics and toxicology, and more specifically with identifying and predicting inter-patient differences in response to drugs in order to achieve superior efficacy and safety in selected patient populations.

It is further concerned with the genetic basis of inter-patient variation in response to therapy, including drug therapy, and with methods for determining and exploiting such differences to improve medical outcomes. Specifically, this invention describes the identification of genes and gene sequence variances useful in the field of therapeutics for optimizing efficacy and safety of drug therapy by allowing prediction of pharmacokinetic and/or toxicologic behavior of specific drugs in specific patients. Relevant pharmacokinetic processes include absorption, distribution, metabolism and excretion. Relevant toxicological processes include both dose related and idiosyncratic adverse reactions to drugs, including, for example, hepatotoxicity, blood dyscrasias and immunological reactions.

It is further concerned with the genetic basis of inter-patient variation in response to therapy, including drug therapy. Specifically, this invention describes the identification of gene sequence variances useful in the field of therapeutics for optimizing efficacy and safety of drug therapy. These variances may be useful
5 either during the drug development process or in guiding the optimal use of already approved compounds. DNA sequence variances in candidate genes (i.e. genes that may plausibly affect the action of a drug) are tested in clinical trials, leading to the establishment of diagnostic tests useful for improving the development of new pharmaceutical products and/or the more effective use of existing pharmaceutical
10 products. Methods for identifying genetic variances and determining their utility in the selection of optimal therapy for specific patients are also described. In general, the invention relates to methods for identifying patient population subsets that respond to drug therapy with either therapeutic benefit or side effects (i.e. symptomatology prompting concern about safety or other unwanted signs or
15 symptoms).

This broad range of pharmacological interactions with receptors, transporters, enzymes and other proteins which are differentially expressed in different populations of cells, e.g., in the CNS, has implications for the design of experiments to identify genetic determinants of drug response. In particular, because
20 of the broad pharmacological interactions of compounds being developed as CNS drugs it may be necessary to study the effect of DNA sequence variances in a number of different sets of genes (belonging to different biochemical pathways) in order to identify a sequence variance or set of variances responsible for interpatient variation in drug response. Methods are described herein for identifying relevant
25 DNA sequence variances and associating them with drug response phenotypes.

While the complexity of CNS physiology creates challenges for pharmacogenetic studies, it is also the case that the pharmacological treatment of CNS diseases provides broad scope for the methods of this invention, because (i) the hereditary component of many CNS diseases is well established, indicating a major
30 role of genetic (as opposed to environmental) factors in disease etiology, (ii) the molecular pharmacology of CNS drugs is generally well understood, providing a rational basis for selecting genes for pharmacogenetic investigation (iii) the heterogeneous responses of patients to CNS drugs suggests that the factors governing response extend beyond presently understood mechanisms; genetic
35 variation can affect virtually all aspects of pharmacology, and is, for the reasons cited above, likely to account for much of the heterogeneity in drug response. In this application we describe methods for improving the treatment of neurological and psychiatric diseases, movement disorders, neurodegenerative diseases, disorders of

sensation, and cerebrovascular diseases. Specifically, we address the treatment of migraine, pain, epilepsy, schizophrenia, stroke, depression, anxiety, spasticity, Parkinson's disease, dementia, demyelinating disease, amyotrophic lateral sclerosis, and Huntington's disease.

5 Specifically, this invention describes the identification of genes and gene sequence variances useful in the field of therapeutics for optimizing efficacy and safety of drug therapy by allowing prediction of pharmacokinetic and/or toxicologic behavior of specific drugs in specific patients. Relevant pharmacokinetic processes include absorption, distribution, metabolism and excretion. Relevant toxicological
10 processes include both dose related and idiosyncratic adverse reactions to drugs, including, for example, hepatotoxicity, blood dyscrasias and immunological reactions.

The invention also describes methods for establishing diagnostic tests useful in (i) the development of, (ii) obtaining regulatory approval for and (iii) safe and
15 efficacious clinical use of pharmaceutical products. These variances may be useful either during the drug development process or in guiding the optimal use of already approved compounds. DNA sequence variances in candidate genes (i.e. genes that may plausibly affect the action of a drug) are tested in clinical trials, leading to the establishment of diagnostic tests useful for improving the development of new
20 pharmaceutical products and/or the more effective use of existing pharmaceutical products. Methods for identifying genetic variances and determining their utility in the selection of optimal therapy for specific patients are also described. In general, the invention relates to methods for identifying and dealing effectively with the genetic sources of interpatient variation in drug response, including both variable
25 efficacy as determined by pharmacokinetic variability and variable toxicity as determined by pharmacokinetic factors or by other genetic factors (e.g. factors responsible for idiosyncratic drug response).

This application is directed also to diseases in which abnormal function of the immune system or the inflammatory response is part of the disease process, or in
30 which modulation of immune or inflammatory function is being tested as a therapeutic intervention. Specifically we address the treatment of arthritis, chronic obstructive pulmonary disease, autoimmune disease, transplantation, pain associated with inflammation, psoriasis, atherosclerosis, asthma, inflammatory bowel disease, and hepatitis.

35 In this application we further describe methods for improving the treatment of endocrine and metabolic diseases. Specifically, we address the treatment of diabetes mellitus and the related metabolic syndrome X, diabetes insipidus, obesity, contraception and infertility, osteoporosis, acne, and alopecia. The methods of this

invention are also relevant to devising effective genetic approaches to drug development for endocrine diseases of pituitary, thyroid, parathyroid, adrenal, gonads and secondary sex tissues.

While the complexity of cardiovascular and renal physiology creates
5 challenges for pharmacogenetic studies (e.g. selecting the right genes to study, selecting the relevant DNA sequence variances within those genes, constructing sound genetic statistical tests, etc.), it is also the case that the pharmacological treatment of cardiovascular and renal diseases provides broad scope for the methods of this invention, because (i) the hereditary component of many cardiovascular and
10 renal diseases is well established, indicating a major role of genetic (as opposed to environmental) factors in disease etiology, (ii) the molecular pharmacology of cardiovascular and renal drugs is generally well understood, providing a rational basis for selecting genes for pharmacogenetic investigation (iii) the heterogeneous responses of patients to cardiovascular and renal drugs suggests that the factors
15 governing response extend beyond presently understood mechanisms; genetic variation can affect virtually all aspects of pharmacology, and are, for the reasons cited above, likely to account for much of the heterogeneity in drug response. In this application we describe methods for improving the treatment of cardiovascular and renal diseases. Specifically, we address the treatment of anemia, angina (including
20 coronary artery atherosclerosis), arrhythmias, hypertension, hypotension, myocardial ischemia, heart failure, thrombosis, renal diseases, restenosis, and peripheral vascular disease (including atherosclerosis). The methods of this invention are also relevant to devising effective genetic approaches to drug development for other cardiovascular and renal diseases.

25 Described in the Examples and Tables are pathways, genes and gene sequence variances useful in the genetic analysis of treatment response for each of these diseases, and exemplary compounds being developed to treat each of these diseases, the use of which may be improved by genetic analysis of the type described herein.

30 The inventors have determined that the identification of gene sequence variances in genes that may be involved in drug action are useful for determining whether genetic variances account for variable drug efficacy and safety and for determining whether a given drug or other therapy may be safe and effective in an individual patient. Provided in this invention are identifications of genes and
35 sequence variances which can be useful in connection with predicting differences in response to treatment and selection of appropriate treatment of a disease or condition. A target gene and variances are useful, for example, in pharmacogenetic association studies and diagnostic tests to improve the use of certain drugs or other

therapies including, but not limited to, the drug classes and specific drugs identified in the 1999 Physicians' Desk Reference (53rd edition), Medical Economics Data, 1998, the 1995 United States Pharmacopeia XXIII National Formulary XVIII, Interpharm Press, 1994, Tables 24-68 or other sources as described below.

5 The terms "disease" or "condition" are commonly recognized in the art and designate the presence of signs and/or symptoms in an individual or patient that are generally recognized as abnormal. Diseases or conditions may be diagnosed and categorized based on pathological changes. Signs may include any objective evidence of a disease such as changes that are evident by physical examination of a
10 patient or the results of diagnostic tests which may include, among others, laboratory tests to determine the presence of DNA sequence variances or variant forms of certain genes in a patient. Symptoms are subjective evidence of disease or a patients condition, i.e. the patients perception of an abnormal condition that differs from normal function, sensation, or appearance, which may include, without limitations,
15 physical disabilities, morbidity, pain, and other changes from the normal condition experienced by an individual. Various diseases or conditions include, but are not limited to; those categorized in standard textbooks of medicine including, without limitation, textbooks of nutrition, allopathic, homeopathic, and osteopathic
20 medicine. In certain aspects of this invention, the disease or condition is selected from the group consisting of the types of diseases listed in standard texts such as Harrison's Principles of Internal Medicine (14th Ed) by Anthony S. Fauci, Eugene Braunwald, Kurt J. Isselbacher, et al. (Editors), McGraw Hill, 1997, or Robbins
25 Pathologic Basis of Disease (6th edition) by Ramzi S. Cotran, Vinay Kumar, Tucker Collins & Stanley L. Robbins, W B Saunders Co., 1998, or the Diagnostic and Statistical Manual of Mental Disorders: DSM-IV (4th edition), American Psychiatric Press, 1994, or other texts described below. Examples for this invention include, neoplastic disorders such as cancer, amyotrophic lateral sclerosis, anxiety, dementia, depression, epilepsy, Huntington's disease, migraine, demyelinating disease, multiple sclerosis, pain, Parkinson's disease, schizophrenia, spasticity, psychoses,
30 and stroke, drug-induced diseases, disorders, or toxicities consisting of blood dyscrasias, cutaneous toxicities, systemic toxicities, central nervous system toxicities, hepatic toxicities, cardiovascular toxicities, pulmonary toxicities, and renal toxicities, arthritis, chronic obstructive pulmonary disease, autoimmune disease, transplantation, pain associated with inflammation, psoriasis,
35 atherosclerosis, asthma, inflammatory bowel disease, and hepatitis, diabetes mellitus, metabolic syndrome X, diabetes insipidus, obesity, contraception, infertility, hormonal insufficiency related to aging, osteoporosis, acne, alopecia, adrenal dysfunction, thyroid dysfunction, and parathyroid dysfunction, anemia,

angina, arrhythmia, hypertension, hypothermia, ischemia, heart failure, thrombosis, renal disease, restenosis, and peripheral vascular disease

In connection with the methods of this invention, unless otherwise indicated, the term "suffering from a disease or condition" means that a person is either
5 presently subject to the signs and symptoms, or is more likely to develop such signs and symptoms than a normal person in the population. Thus, for example, a person suffering from a condition can include a developing fetus, a person subject to a treatment or environmental condition which enhances the likelihood of developing the signs or symptoms of a condition, or a person who is being given or will be
10 given a treatment which increase the likelihood of the person developing a particular condition. For example, tardive dyskinesia is associated with long-term use of anti-psychotics; dyskinesias, paranoid ideation, psychotic episodes and depression have been associated with use of L-dopa in Parkinson's disease; (and dizziness, diplopia, ataxia, sedation, impaired mentation, weight gain, and other undesired effects have
15 been described for various anticonvulsant therapies. Thus, methods of the present invention which relate to treatments of patients (e.g., methods for selecting a treatment, selecting a patient for a treatment, and methods of treating a disease or condition in a patient) can include primary treatments directed to a presently active disease or condition, secondary treatments which are intended to cause a biological effect relevant to a primary treatment, and prophylactic treatments intended to delay,
20 reduce, or prevent the development of a disease or condition, as well as treatments intended to cause the development of a condition different from that which would have been likely to develop in the absence of the treatment.

The term "therapy" refers to a process which is intended to produce a
25 beneficial change in the condition of a mammal, e.g., a human, often referred to as a patient. A beneficial change can, for example, include one or more of: restoration of function, reduction of symptoms, limitation or retardation of progression of a disease, disorder, or condition or prevention, limitation or retardation of deterioration of a patient's condition, disease or disorder. Such therapy can involve,
30 for example, nutritional modifications, administration of radiation, administration of a drug, behavioral modifications, and combinations of these, among others.

The term "drug" as used herein refers to a chemical entity or biological product, or combination of chemical entities or biological products, administered to a person to treat or prevent or control a disease or condition. The chemical entity or
35 biological product is preferably, but not necessarily a low molecular weight compound, but may also be a larger compound, for example, an oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNazymes, glycoproteins, lipoproteins, and

modifications and combinations thereof. A biological product is preferably a monoclonal or polyclonal antibody or fragment thereof such as a variable chain fragment; cells; or an agent or product arising from recombinant technology, such as, without limitation, a recombinant protein, recombinant vaccine, or DNA construct developed for therapeutic, e.g., human therapeutic, use. The term "drug" may include, without limitation, compounds that are approved for sale as pharmaceutical products by government regulatory agencies (e.g., U.S. Food and Drug Administration (USFDA or FDA), European Medicines Evaluation Agency (EMEA), and a world regulatory body governing the International Conference of Harmonization (ICH) rules and guidelines), compounds that do not require approval by government regulatory agencies, food additives or supplements including compounds commonly characterized as vitamins, natural products, and completely or incompletely characterized mixtures of chemical entities including natural compounds or purified or partially purified natural products. The term "drug" as used herein is synonymous with the terms "medicine", "pharmaceutical product", or "product". Most preferably the drug is approved by a government agency for treatment of a specific disease or condition.

A "low molecular weight compound" has a molecular weight <5,000 Da, more preferably <2500 Da, still more preferably <1000 Da, and most preferably <700 Da.

Those familiar with drug use in medical practice will recognize that regulatory approval for drug use is commonly limited to approved indications, such as to those patients afflicted with a disease or condition for which the drug has been shown to be likely to produce a beneficial effect in a controlled clinical trial. Unfortunately, it has generally not been possible with current knowledge to predict which patients will have a beneficial response, with the exception of certain diseases such as bacterial infections where suitable laboratory methods have been developed. Likewise, it has generally not been possible to determine in advance whether a drug will be safe in a given patient. Regulatory approval for the use of most drugs is limited to the treatment of selected diseases and conditions. The descriptions of approved drug usage, including the suggested diagnostic studies or monitoring studies, and the allowable parameters of such studies, are commonly described in the "label" or "insert" which is distributed with the drug. Such labels or inserts are preferably required by government agencies as a condition for marketing the drug and are listed in common references such as the Physicians Desk Reference (PDR). These and other limitations or considerations on the use of a drug are also found in medical journals, publications such as pharmacology, pharmacy or medical

textbooks including, without limitation, textbooks of nutrition, allopathic, homeopathic, and osteopathic medicine.

Many widely used drugs are effective in a minority of patients receiving the drug, particularly when one controls for the placebo effect. For example, the PDR shows that about 45% of patients receiving Cognex (tacrine hydrochloride) for Alzheimer's disease show no change or minimal worsening of their disease, as do about 68% of controls (including about 5% of controls who were much worse). About 58% of Alzheimer's patients receiving Cognex were minimally improved, compared to about 33% of controls, while about 2% of patients receiving Cognex were much improved compared to about 1% of controls. Thus a tiny fraction of patients had a significant benefit. Response to treatments for amyotrophic lateral sclerosis are likewise minimal.

Thus, in a first aspect, the invention provides a method for selecting a treatment for a patient suffering from a disease or condition by determining whether or not a gene or genes in cells of the patient (in some cases including both normal and disease cells, such as cancer cells) contain at least one sequence variance which is indicative of the effectiveness of the treatment of the disease or condition. The gene or genes (along with exemplary variances) are specified herein, in Tables 1-6, 12-17, and 18-23. Preferably the at least one variance includes a plurality of variances which may provide a haplotype or haplotypes. Preferably the joint presence of the plurality of variances is indicative of the potential effectiveness or safety of the treatment in a patient having such plurality of variances. The plurality of variances may each be indicative of the potential effectiveness of the treatment, and the effects of the individual variances may be independent or additive, or the plurality of variances may be indicative of the potential effectiveness if at least 2, 3, 4, or more appear jointly. The plurality of variances may also be combinations of these relationships. The plurality of variances may include variances from one, two, three or more gene loci.

In preferred embodiments of aspects of the invention involving genes relating to psychiatric or neurological disease or related conditions or the other diseases or conditions identified herein, or to pharmacological responses to compounds used to treat such diseases or conditions, the gene product is involved in a function as described in the Background of the Invention or otherwise described herein.

In some cases, the selection of a method of treatment, i.e., a therapeutic regimen, may incorporate selection of one or more from a plurality of medical therapies. Thus, the selection may be the selection of a method or methods which is/are more effective or less effective than certain other therapeutic regimens (with

either having varying safety parameters). Likewise or in combination with the preceding selection, the selection may be the selection of a method or methods, which is safer than certain other methods of treatment in the patient.

The selection may involve either positive selection or negative selection or both, meaning that the selection can involve a choice that a particular method would be an appropriate method to use and/or a choice that a particular method would be an inappropriate method to use. Thus, in certain embodiments, the presence of the at least one variance is indicative that the treatment will be effective or otherwise beneficial (or more likely to be beneficial) in the patient. Stating that the treatment will be effective means that the probability of beneficial therapeutic effect is greater than in a person not having the appropriate presence or absence of particular variances. In other embodiments, the presence of the at least one variance is indicative that the treatment will be ineffective or contra-indicated for the patient. For example, a treatment may be contra-indicated if the treatment results, or is more likely to result, in undesirable side effects, or an excessive level of undesirable side effects. A determination of what constitutes excessive side-effects will vary, for example, depending on the disease or condition being treated, the availability of alternatives, the expected or experienced efficacy of the treatment, and the tolerance of the patient. As for an effective treatment, this means that it is more likely that desired effect will result from the treatment administration in a patient with a particular variance or variances than in a patient who has a different variance or variances. Also in preferred embodiments, the presence of the at least one variance is indicative that the treatment is both effective and unlikely to result in undesirable effects or outcomes, or vice versa (is likely to have undesirable side effects but unlikely to produce desired therapeutic effects).

In reference to response to a treatment, the term "tolerance" refers to the ability of a patient to accept a treatment, based, e.g., on deleterious effects and/or effects on lifestyle. Frequently, the term principally concerns the patients perceived magnitude of deleterious effects such as nausea, weakness, dizziness, and diarrhea, among others. Such experienced effects can, for example, be due to general or cell-specific toxicity, activity on non-target cells, cross-reactivity on non-target cellular constituents (non-mechanism based), and/or side effects of activity on the target cellular substituents (mechanism based), or the cause of toxicity may not be understood. In any of these circumstances one may identify an association between the undesirable effects and variances in specific genes.

Adverse responses to drugs constitute a major medical problem, as shown in two recent meta-analyses (Lazarou, J. et al, Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies, JAMA 279:1200-1205,

1998; Bonn, Adverse drug reactions remain a major cause of death, Lancet 351:1183, 1998). An estimated 2.2 million hospitalized patients in the United States had serious adverse drug reactions in 1994, with an estimated 106,000 deaths (Lazarou et al.). To the extent that some of these adverse events are due to genetically encoded biochemical diversity among patients in pathways that effect drug action, the identification of variances that are predictive of such effects will allow for more effective and safer drug use.

In embodiments of this invention, the variance or variant form or forms of a gene is/are associated with a specific response to a drug. The frequency of a specific variance or variant form of the gene may correspond to the frequency of an efficacious response to administration of a drug. Alternatively, the frequency of a specific variance or variant form of the gene may correspond to the frequency of an adverse event resulting from administration of a drug. Alternatively the frequency of a specific variance or variant form of a gene may not correspond closely with the frequency of a beneficial or adverse response, yet the variance may still be useful for identifying a patient subset with high response or toxicity incidence because the variance may account for only a fraction of the patients with high response or toxicity. In such a case the preferred course of action is identification of a second or third or additional variances that permit identification of the patient groups not usefully identified by the first variance. Preferably, the drug will be effective in more than 20% of individuals with one or more specific variances or variant forms of the gene, more preferably in 40% and most preferably in >60%. In other embodiments, the drug will be toxic or create clinically unacceptable side effects in more than 10% of individuals with one or more variances or variant forms of the gene, more preferably in >30%, more preferably in >50%, and most preferably in >70% or in more than 90%.

Also in other embodiments, the method of selecting a treatment includes eliminating or excluding a treatment, where the presence or absence of the at least one variance is indicative that the treatment will be ineffective or contra-indicated, e.g., would result in excessive weight gain. In other preferred embodiments, in cases in which undesirable side-effects may occur or are expected to occur from a particular therapeutic treatment, the selection of a method of treatment can include identifying both a first and second treatment, where the first treatment is effective to treat the disease or condition, and the second treatment reduces a deleterious effect or enhance efficacy of the first treatment.

The phrase "eliminating a treatment" (similarly for excluding a treatment) refers to removing a possible treatment from consideration, e.g., for use with a particular patient based on the presence or absence of a particular variance(s) in one

or more genes in cells of that patient, or to stopping the administration of a treatment.

Usually, the treatment will involve the administration of a compound preferentially active or safe in patients with a form or forms of a gene, where the gene is one identified herein. The administration may involve a combination of compounds. Thus, in preferred embodiments, the method involves identifying such an active compound or combination of compounds, where the compound is less active or is less safe or both when administered to a patient having a different form of the gene.

Also in preferred embodiments, the method of selecting a treatment involves selecting a method of administration of a compound, combination of compounds, or pharmaceutical composition, for example, selecting a suitable dosage level and/or frequency of administration, and/or mode of administration of a compound. The method of administration can be selected to provide better, preferably maximum therapeutic benefit. In this context, "maximum" refers to an approximate local maximum based on the parameters being considered, not an absolute maximum.

Also in this context, a "suitable dosage level" refers to a dosage level which provides a therapeutically reasonable balance between pharmacological effectiveness and deleterious effects. Often this dosage level is related to the peak or average serum levels resulting from administration of a drug at the particular dosage level.

Similarly, a "frequency of administration" refers to how often in a specified time period a treatment is administered, e.g., once, twice, or three times per day, every other day, once per week, etc. For a drug or drugs, the frequency of administration is generally selected to achieve a pharmacologically effective average or peak serum level without excessive deleterious effects (and preferably while still being able to have reasonable patient compliance for self-administered drugs). Thus, it is desirable to maintain the serum level of the drug within a therapeutic window of concentrations for the greatest percentage of time possible without such deleterious effects as would cause a prudent physician to reduce the frequency of administration for a particular dosage level.

A particular gene or genes can be relevant to the treatment of more than one disease or condition, for example, the gene or genes can have a role in the initiation, development, course, treatment, treatment outcomes, or health-related quality of life outcomes of a number of different diseases, disorders, or conditions. Thus, in preferred embodiments, the disease or condition or treatment of the disease or condition is any which involves a gene from the gene list described herein as Tables 1-6, 12-17, and 18-23.

Determining the presence of a particular variance or plurality of variances in a particular gene in a patient can be performed in a variety of ways. In preferred embodiments, the detection of the presence or absence of at least one variance involves amplifying a segment of nucleic acid including at least one of the at least one variances. Preferably a segment of nucleic acid to be amplified is 500 nucleotides or less in length, more preferably 100 nucleotides or less, and most preferably 45 nucleotides or less. Also, preferably the amplified segment or segments includes a plurality of variances, or a plurality of segments of a gene or of a plurality of genes. In other embodiments, e.g., where a haplotype is to be determined, the segment of nucleic acid is at least 500 nucleotides in length, or at least 2 kb in length, or at least 5 kb in length.

In preferred embodiments, determining the presence of a set of variances in a specific gene related to treatment of disease, disorders, or dysfunctions or other related genes, or genes listed in Tables 1-6, 12-17, and 18-23, includes a haplotyping test that requires allele specific amplification of a large DNA segment of no greater than 25,000 nucleotides, preferably no greater than 10,000 nucleotides and most preferably no greater than 5,000 nucleotides. Alternatively one allele may be enriched by methods other than amplification prior to determining genotypes at specific variant positions on the enriched allele as a way of determining haplotypes. Preferably the determination of the presence or absence of a haplotype involves determining the sequence of the variant sites by methods such as chain terminating DNA sequencing or minisequencing, or by oligonucleotide hybridization or by mass spectrometry. For the use of mass spectrometry, the method can involve detection of the mass of a fragment or fragments and can further involve inferring the genotype (e.g., the specific variance at a site) from the masses determined.

The term "genotype" in the context of this invention refers to the alleles present in DNA from a subject or patient, where an allele can be defined by the particular nucleotide(s) present in a nucleic acid sequence at a particular site(s). Often a genotype is the nucleotide(s) present at a single polymorphic site known to vary in the human population.

In preferred embodiments, the detection of the presence or absence of the at least one variance involves contacting a nucleic acid sequence corresponding to one of the genes identified above or a product of such a gene with a probe. The probe is able to distinguish a particular form of the gene or gene product or the presence or a particular variance or variances, e.g., by differential binding or hybridization. Thus, exemplary probes include nucleic acid hybridization probes, peptide nucleic acid probes, nucleotide-containing probes which also contain at least one nucleotide analog, and antibodies, e.g., monoclonal antibodies, and other probes as discussed

herein. Those skilled in the art are familiar with the preparation of probes with particular specificities. Those skilled in the art will recognize that a variety of variables can be adjusted to optimize the discrimination between two variant forms of a gene, including changes in salt concentration, temperature, pH and addition of various compounds that affect the differential affinity of GC vs. AT base pairs, such as tetramethyl ammonium chloride. (See Current Protocols in Molecular Biology by F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.D. Seidman, K. Struhl, and V.B. Chanda (editors, John Wiley & Sons.)

In other preferred embodiments, determining the presence or absence of the at least one variance involves sequencing at least one nucleic acid sample. The sequencing involves sequencing of a portion or portions of a gene and/or portions of a plurality of genes which includes at least one variance site, and may include a plurality of such sites. Preferably, the portion is 500 nucleotides or less in length, more preferably 100 nucleotides or less, and most preferably 45 nucleotides or less in length. Such sequencing can be carried out by various methods recognized by those skilled in the art, including use of dideoxy termination methods (e.g., using dye-labeled dideoxy nucleotides) and the use of mass spectrometric methods. In addition, mass spectrometric methods may be used to determine the nucleotide present at a variance site. In preferred embodiments in which a plurality of variances is determined, the plurality of variances can constitute a haplotype or collection of haplotypes. Preferably the methods for determining genotypes or haplotypes are designed to be sensitive to all the common genotypes or haplotypes present in the population being studied (for example, a clinical trial population).

The terms "variant form of a gene", "form of a gene", or "allele" refer to one specific form of a gene in a population, the specific form differing from other forms of the same gene in the sequence of at least one, and frequently more than one, variant sites within the sequence of the gene. The sequences at these variant sites that differ between different alleles of the gene are termed "gene sequence variances" or "variances" or "variants". The term "alternative form" refers to an allele that can be distinguished from other alleles by having distinct variances at least one, and frequently more than one, variant sites within the gene sequence. Other terms known in the art to be equivalent include mutation and polymorphism, although mutation is often used to refer to an allele associated with a deleterious phenotype. In preferred aspects of this invention, the variances are selected from the group consisting of the variances listed in the variance tables herein or in a patent or patent application referenced and incorporated by reference in this disclosure. In the methods utilizing variance presence or absence, reference to the presence of a variance or variances means particular variances, i.e., particular nucleotides at

particular polymorphic sites, rather than just the presence of any variance in the gene.

Variances occur in the human genome at approximately one in every 500 – 1,000 bases within the human genome when two alleles are compared. When multiple alleles from unrelated individuals are compared the density of variant sites increases as different individuals, when compared to a reference sequence, will often have sequence variances at different sites. At most variant sites there are only two alternative nucleotides involving the substitution of one base for another or the insertion/deletion of one or more nucleotides. Within a gene there may be several variant sites. Variant forms of the gene or alternative alleles can be distinguished by the presence of alternative variances at a single variant site, or a combination of several different variances at different sites (haplotypes).

It is estimated that there are 3,300,000,000 bases in the sequence of a single haploid human genome. All human cells except germ cells are normally diploid. Each gene in the genome may span 100-10,000,000 bases of DNA sequence or 100-20,000 bases of mRNA. It is estimated that there are between 60,000 and 150,000 genes in the human genome. The "identification" of genetic variances or variant forms of a gene involves the discovery of variances that are present in a population. The identification of variances is required for development of a diagnostic test to determine whether a patient has a variant form of a gene that is known to be associated with a disease, condition, or predisposition or with the efficacy or safety of the drug. Identification of previously undiscovered genetic variances is distinct from the process of "determining" the status of known variances by a diagnostic test (often referred to as genotyping). The present invention provides exemplary variances in genes listed in the gene tables, as well as methods for discovering additional variances in those genes and a comprehensive written description of such additional possible variances. Also described are methods for DNA diagnostic tests to determine the DNA sequence at a particular variant site or sites.

The process of "identifying" or discovering new variances involves comparing the sequence of at least two alleles of a gene, more preferably at least 10 alleles and most preferably at least 50 alleles (keeping in mind that each somatic cell has two alleles). The analysis of large numbers of individuals to discover variances in the gene sequence between individuals in a population will result in detection of a greater fraction of all the variances in the population. Preferably the process of identifying reveals whether there is a variance within the gene; more preferably identifying reveals the location of the variance within the gene; more preferably identifying provides knowledge of the sequence of the nucleic acid sequence of the

variance, and most preferably identifying provides knowledge of the combination of different variances that comprise specific variant forms of the gene (referred to as alleles). In identifying new variances it is often useful to screen different population groups based on racial, ethnic, gender, and/or geographic origin because particular variances may differ in frequency between such groups. It may also be useful to screen DNA from individuals with a particular disease or condition of interest because they may have a higher frequency of certain variances than the general population.

The process of genotyping involves using diagnostic tests for specific variances that have already been identified. It will be apparent that such diagnostic tests can only be performed after variances and variant forms of the gene have been identified. Identification of new variances can be accomplished by a variety of methods, alone or in combination, including, for example, DNA sequencing, SSCP, heteroduplex analysis, denaturing gradient gel electrophoresis (DGGE), heteroduplex cleavage (either enzymatic as with T4 Endonuclease 7, or chemical as with osmium tetroxide and hydroxylamine), computational methods (described herein), and other methods described herein as well as others known to those skilled in the art. (See, for example: Cotton, R.G.H., Slowly but surely towards better scanning for mutations, Trends in Genetics 13(2): 43-6, 1997 or Current Protocols in Human Genetics by N.C. Dracoli, J.L. Haines, B.R. Korf, D.T. Moir, C.C. Morton, C.E. Seidman, D.R. Smith, and A. Boyle (editors), John Wiley & Sons.)

In the context of this invention, the term "analyzing a sequence" refers to determining at least some sequence information about the sequence, e.g., determining the nucleotides present at a particular site or sites in the sequence, particularly sites that are known to vary in a population, or determining the base sequence of all or of a portion of the particular sequence.

In the context of this invention, the term "haplotype" refers to a *cis* arrangement of two or more polymorphic nucleotides, i.e., variances, on a particular chromosome, e.g., in a particular gene. The haplotype preserves information about the phase of the polymorphic nucleotides – that is, which set of variances were inherited from one parent, and which from the other. A genotyping test does not provide information about phase. For example, an individual heterozygous at nucleotide 25 of a gene (both A and C are present) and also at nucleotide 100 (both G and T are present) could have haplotypes 25A – 100G and 25C – 100T, or alternatively 25A – 100T and 25C – 100G. Only a haplotyping test can discriminate these two cases definitively.

The terms "variances", "variants" and "polymorphisms", as used herein, may also refer to a set of variances, haplotypes or a mixture of the two, unless otherwise

indicated. Further, the term variance, variant or polymorphism (singular), as used herein, also encompasses a haplotype unless otherwise indicated. This usage is intended to minimize the need for cumbersome phrases such as: "...measure correlation between drug response and *a variance, variances, haplotype, haplotypes or a combination of variances and haplotypes...*", throughout the application.

Instead, the italicized text in the foregoing sentence can be represented by the word "variance", "variant" or "polymorphism". Similarly, the term genotype, as used herein, means a procedure for determining the status of one or more variances in a gene, including a set of variances comprising a haplotype. Thus phrases such as "...genotype a patient..." refer to determining the status of one or more variances, including a set of variances for which phase is known (i.e. a haplotype).

In preferred embodiments of this invention, the frequency of the variance or variant form of the gene in a population is known. Measures of frequency known in the art include "allele frequency", namely the fraction of genes in a population that have one specific variance or set of variances. The allele frequencies for any gene should sum to 1. Another measure of frequency known in the art is the "heterozygote frequency" namely, the fraction of individuals in a population who carry two alleles, or two forms of a particular variance or variant form of a gene, one inherited from each parent. Alternatively, the number of individuals who are homozygous for a particular form of a gene may be a useful measure. The relationship between allele frequency, heterozygote frequency, and homozygote frequency is described for many genes by the Hardy-Weinberg equation, which provides the relationship between allele frequency, heterozygote frequency and homozygote frequency in a freely breeding population at equilibrium. Most human variances are substantially in Hardy-Weinberg equilibrium. In a preferred aspect of this invention, the allele frequency, heterozygote frequency, and homozygote frequencies are determined experimentally. Preferably a variance has an allele frequency of at least 0.01, more preferably at least 0.05, still more preferably at least 0.10. However, the allele may have a frequency as low as 0.001 if the associated phenotype is, for example, a rare form of toxic reaction to a treatment or drug. Beneficial responses may also be rare.

In this regard, "population" refers to a defined group of individuals or a group of individuals with a particular disease or condition or individuals that may be treated with a specific drug identified by, but not limited to geographic, ethnic, race, gender, and/or cultural indices. In most cases a population will preferably encompass at least ten thousand, one hundred thousand, one million, ten million, or more individuals, with the larger numbers being more preferable. In preferred embodiments of this invention, the population refers to individuals with a specific

disease or condition that may be treated with a specific drug. In embodiments of this invention, the allele frequency, heterozygote frequency, or homozygote frequency of a specific variance or variant form of a gene is known. In preferred embodiments of this invention, the frequency of one or more variances that may predict response to a treatment is determined in one or more populations using a diagnostic test.

It should be emphasized that it is currently not generally practical to study an entire population to establish the association between a specific disease or condition or response to a treatment and a specific variance or variant form of a gene. Such studies are preferably performed in controlled clinical trials using a limited number of patients that are considered to be representative of the population with the disease. Since drug development programs are generally targeted at the largest possible population, the study population will generally consist of men and women, as well as members of various racial and ethnic groups, depending on where the clinical trial is being performed. This is important to establish the efficacy of the treatment in all segments of the population.

In the context of this invention, the term "probe" refers to a molecule that detectably distinguishes between target molecules differing in structure. Detection can be accomplished in a variety of different ways depending on the type of probe used and the type of target molecule. Thus, for example, detection may be based on discrimination of activity levels of the target molecule, but preferably is based on detection of specific binding. Examples of such specific binding include antibody binding and nucleic acid probe hybridization. Thus, for example, probes can include enzyme substrates, antibodies and antibody fragments, and nucleic acid hybridization probes. Thus, in preferred embodiments, the detection of the presence or absence of the at least one variance involves contacting a nucleic acid sequence which includes a variance site with a probe, preferably a nucleic acid probe, where the probe preferentially hybridizes with a form of the nucleic acid sequence containing a complementary base at the variance site as compared to hybridization to a form of the nucleic acid sequence having a non-complementary base at the variance site, where the hybridization is carried out under selective hybridization conditions. Such a nucleic acid hybridization probe may span two or more variance sites. Unless otherwise specified, a nucleic acid probe can include one or more nucleic acid analogs, labels or other substituents or moieties so long as the base-pairing function is retained.

As is generally understood, administration of a particular treatment, e.g., administration of a therapeutic compound or combination of compounds, is chosen depending on the disease or condition that is to be treated. Thus, in certain preferred

embodiments, the disease or condition is one for which administration of a treatment is expected to provide a therapeutic benefit; in certain embodiments, the compound is a compound identified herein, e.g., in a drug table (Tables 24-68).

As used herein, the terms "effective" and "effectiveness" includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the treatment to result in a desired biological effect in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (often referred to as side-effects) resulting from administration of the treatment. On the other hand, the term "ineffective" indicates that a treatment does not provide sufficient pharmacological effect to be therapeutically useful, even in the absence of deleterious effects, at least in the unstratified population. (Such a treatment may be ineffective in a subgroup that can be identified by the presence of one or more sequence variances or alleles.) "Less effective" means that the treatment results in a therapeutically significant lower level of pharmacological effectiveness and/or a therapeutically greater level of adverse physiological effects, e.g., greater liver toxicity.

Thus, in connection with the administration of a drug, a drug which is "effective against" a disease or condition indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as a improvement of symptoms, a cure, a reduction in disease load, reduction in tumor mass or cell numbers, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating the particular type of disease or condition.

Effectiveness is measured in a particular population. In conventional drug development the population is generally every subject who meets the enrollment criteria (i.e. has the particular form of the disease or condition being treated). It is an aspect of the present invention that segmentation of a study population by genetic criteria can provide the basis for identifying a subpopulation in which a drug is effective against the disease or condition being treated.

The term "deleterious effects" refers to physical effects in a patient caused by administration of a treatment which are regarded as medically undesirable. Thus, for example, deleterious effects can include a wide spectrum of toxic effects injurious to health such as death of normally functioning cells when only death of diseased cells is desired, nausea, fever, inability to retain food, dehydration, damage to critical organs such as arrhythmias, renal tubular necrosis, fatty liver, or pulmonary fibrosis leading to coronary, renal, hepatic, or pulmonary insufficiency among many others. In this regard, the term "contra-indicated" means that a treatment results in

deleterious effects such that a prudent medical doctor treating such a patient would regard the treatment as unsuitable for administration. Major factors in such a determination can include, for example, availability and relative advantages of alternative treatments, consequences of non-treatment, and permanency of deleterious effects of the treatment.

It is recognized that many treatment methods, e.g., administration of certain compounds or combinations of compounds, may produce side-effects or other deleterious effects in patients. Such effects can limit or even preclude use of the treatment method in particular patients, or may even result in irreversible injury, dysfunction, or death of the patient. Thus, in certain embodiments, the variance information is used to select both a first method of treatment and a second method of treatment. Usually the first treatment is a primary treatment which provides a physiological effect directed against the disease or condition or its symptoms. The second method is directed to reducing or eliminating one or more deleterious effects or enhancing efficacy of the first treatment, e.g., to reduce a general toxicity or to reduce a side effect of the primary treatment. Thus, for example, the second method can be used to allow use of a greater dose or duration of the first treatment, or to allow use of the first treatment in patients for whom the first treatment would not be tolerated or would be contra-indicated in the absence of a second method to reduce deleterious effects or to potentiate the effectiveness of the first treatment.

In a related aspect, the invention concerns a method for providing a correlation between a patient genotype and effectiveness of a treatment, by determining the presence or absence of a particular known variance or variances in cells of a patient for a gene from Tables 1-6, 12-17, and 18-23, or other gene related to neurological disease or other disease identified herein, and providing a result indicating the expected effectiveness of a treatment for a disease or condition. The result may be formulated by comparing the genotype of the patient with a list of variances indicative of the effectiveness of a treatment, e.g., administration of a drug described herein. The determination may be by methods as described herein or other methods known to those skilled in the art.

In a related aspect, the invention provides a method for selecting a method of treatment for a patient suffering from a disease or condition as identified herein by comparing at least one variance in at least one gene in the patient, with a list of variances in the gene from Tables 1-6, 12-17, and 18-23, or other gene related to a disease or condition listed herein, which are indicative of the effectiveness of at least one method of treatment. Preferably the comparison involves a plurality of variances or a haplotype indicative of the effectiveness of at least one method of treatment. Also, preferably the list of variances includes a plurality of variances.

Similar to the above aspect, in preferred embodiments the at least one method of treatment involves the administration of a compound effective in at least some patients with a disease or condition; the presence or absence of the at least one variance is indicative that the treatment will be effective in the patient; and/or the presence or absence of the at least one variance is indicative that the treatment will be ineffective or contra-indicated in the patient; and/or the treatment is a first treatment and the presence or absence of the at least one variance is indicative that a second treatment will be beneficial to reduce a deleterious effect of or potentiate the effectiveness of the first treatment; and/or the at least one treatment is a plurality of methods of treatment. For a plurality of treatments, preferably the selecting involves determining whether any of the methods of treatment will be more effective than at least one other of the plurality of methods of treatment. Yet other embodiments are provided as described for the preceding aspect in connection with methods of treatment using administration of a compound; treatment of various diseases, and variances in particular genes.

In the context of variance information in the methods of this invention, the term "list" refers to one or more, preferably at least 2, 3, 4, 5, 7, or 10 variances that have been identified for a gene of potential importance in accounting for inter-individual variation in treatment response. Preferably there is a plurality of variances for the gene, preferably a plurality of variances for the particular gene. Preferably, the list is recorded in written or electronic form. For example, identified variances of identified genes are recorded for some of the genes in Tables 12-17 and 18-23; additional variances for genes in Tables 1-6 can be readily identified by one skilled in the art using any of a variety of methods. The list may also contain haplotypes, either alone or with other variances.

In addition to the basic method of treatment, often the mode of administration of a given compound as a treatment for a disease or condition in a patient is significant in determining the course and/or outcome of the treatment for the patient. Thus, the invention also provides a method for selecting a method of administration of a compound to a patient suffering from a disease or condition, by determining the presence or absence of at least one variance in cells of the patient in at least one identified gene from Tables 1-6, 12-17, and 18-23, where such presence or absence is indicative of an appropriate method of administration of the compound. Preferably, the selection of a method of treatment (a treatment regimen) involves selecting a dosage level or frequency of administration or route of administration of the compound or combinations of those parameters. In preferred embodiments, two or more compounds are to be administered, and the selecting involves selecting a method of administration for one, two, or more than two of the

compounds, jointly, concurrently, or separately. As understood by those skilled in the art, such plurality of compounds may be used in combination therapy, and thus may be formulated in a single drug, or may be separate drugs administered concurrently, serially, or separately. Other embodiments are as indicated above for
5 selection of second treatment methods, methods of identifying variances, and methods of treatment as described for aspects above.

In another aspect, the invention provides a method for selecting a patient for administration of a method of treatment for a disease or condition, or of selecting a patient for a method of administration of a treatment, by comparing the presence or
10 absence of at least one variance in a gene as identified above in cells of a patient, with a list of variances in the gene, where the presence or absence of the at least one variance is indicative that the treatment or method of administration will be effective in the patient. If the at least one variance is present in the patient's cells, then the patient is selected for administration of the treatment.

15 In preferred embodiments, the disease or the method of treatment is as described in aspects above, specifically including, for example, those described for selecting a method of treatment.

In another aspect, the invention provides a method for identifying a subset of patients with enhanced or diminished response or tolerance to a treatment method or
20 a method of administration of a treatment where the treatment is for a disease or condition in the patient. The method involves correlating one or more variances in one or more genes as identified in aspects above in a plurality of patients with response to a treatment or a method of administration of a treatment. The correlation may be performed by determining the one or more variances in the one
25 or more genes in the plurality of patients and correlating the presence or absence of each of the variances (alone or in various combinations) with the patient's response to treatment. The variances may be previously known to exist or may also be determined in the present method or combinations of prior information and newly determined information may be used. The enhanced or diminished response should
30 be statistically significant, preferably such that $p = 0.10$ or less, more preferably 0.05 or less, and most preferably 0.02 or less. A positive correlation between the presence of one or more variances and an enhanced response to treatment is indicative that the treatment is particularly effective in the group of patients having those variances. A positive correlation of the presence of the one or more variances
35 with a diminished response to the treatment is indicative that the treatment will be less effective in the group of patients having those variances. Such information is useful, for example, for selecting or de-selecting patients for a particular treatment or method of administration of a treatment, or for demonstrating that a group of

patients exists for which the treatment or method of treatment would be particularly beneficial or contra-indicated. Such demonstration can be beneficial, for example, for obtaining government regulatory approval for a new drug or a new use of a drug

In preferred embodiments, the variances are in at least one of the identified
5 genes listed on Tables 1-6, 12-17, and 18-23, or are particular variances described herein. Also, preferred embodiments include drugs, treatments, variance identification or determination, determination of effectiveness, and/or diseases as described for aspects above or otherwise described herein.

In preferred embodiments, the correlation of patient responses to therapy
10 according to patient genotype is carried out in a clinical trial, e.g., as described herein according to any of the variations described. Detailed description of methods for associating variances with clinical outcomes using clinical trials are provided below. Further, in preferred embodiments the correlation of pharmacological effect
15 (positive or negative) to treatment response according to genotype or haplotype in such a clinical trial is part of a regulatory submission to a government agency leading to approval of the drug. Most preferably the compound or compounds would not be approvable in the absence of the genetic information allowing identification of an optimal responder population.

As indicated above, in aspects of this invention involving selection of a
20 patient for a treatment, selection of a method or mode of administration of a treatment, and selection of a patient for a treatment or a method of treatment, the selection may be positive selection or negative selection. Thus, the methods can include eliminating or excluding a treatment for a patient, eliminating or excluding a method or mode of administration of a treatment to a patient, or elimination or
25 exclusion of a patient for a treatment or method of treatment.

Also, in methods involving identification and/or comparison of variances present in a gene of a patient, the methods can involve such identification or comparison for a plurality of genes. Preferably, the genes are functionally related to the same disease or condition, or to the aspect of disease pathophysiology that is
30 being subjected to pharmacological manipulation by the treatment (e.g., a drug), or to the activation or inactivation or elimination of the drug, and more preferably the genes are involved in the same biochemical process or pathway.

In another aspect, the invention provides a method for identifying the forms of a gene in an individual, where the gene is one specified as for aspects above, by
35 determining the presence or absence of at least one variance in the gene. In preferred embodiments, the at least one variance includes at least one variance selected from the group of variances identified in variance tables herein. Preferably, the presence or absence of the at least one variance is indicative of the effectiveness

of a therapeutic treatment in a patient suffering from a disease or condition and having cells containing the at least one variance.

The presence or absence of the variances can be determined in any of a variety of ways as recognized by those skilled in the art. For example, the nucleotide sequence of at least one nucleic acid sequence which includes at least one variance site (or a complementary sequence) can be determined, such as by chain termination methods, hybridization methods or by mass spectrometric methods. Likewise, in preferred embodiments, the determining involves contacting a nucleic acid sequence or a gene product of one of one of the genes with a probe that specifically identifies the presence or absence of a form of the gene. For example, a probe, e.g., a nucleic acid probe, can be used which specifically binds, e.g., hybridizes, to a nucleic acid sequence corresponding to a portion of the gene and which includes at least one variance site under selective binding conditions. As described for other aspects, determining the presence or absence of at least two variances and their relationship on the two gene copies present in a patient can constitute determining a haplotype or haplotypes.

Other preferred embodiments involve variances related to types of treatment, drug responses, diseases, nucleic acid sequences, and other items related to variances and variance determination as described for aspects above.

In yet another aspect, the invention provides a pharmaceutical composition which includes a compound which has a differential effect in patients having at least one copy, or alternatively, two copies of a form of a gene as identified for aspects above and a pharmaceutically acceptable carrier, excipient, or diluent. The composition is adapted to be preferentially effective to treat a patient with cells containing the one, two, or more copies of the form of the gene.

In preferred embodiments of aspects involving pharmaceutical compositions, active compounds, or drugs, the material is subject to a regulatory limitation or restriction on approved uses or indications, e.g., by the U.S. Food and Drug Administration (FDA), limiting approved use of the composition to patients having at least one copy of the particular form of the gene which contains at least one variance. Alternatively, the composition is subject to a regulatory limitation or restriction on approved uses indicating that the composition is not approved for use or should not be used in patients having at least one copy of a form of the gene including at least one variance. Also in preferred embodiments, the composition is packaged, and the packaging includes a label or insert indicating or suggesting beneficial therapeutic approved use of the composition in patients having one or two copies of a form of the gene including at least one variance. Alternatively, the label or insert limits approved use of the composition to patients having zero or one

or two copies of a form of the gene including at least one variance. The latter embodiment would be likely where the presence of the at least one variance in one or two copies in cells of a patient means that the composition would be ineffective or deleterious to the patient. Also in preferred embodiments, the composition is indicated for use in treatment of a disease or condition which is one of those identified for aspects above. Also in preferred embodiments, the at least one variance includes at least one variance from those identified herein.

The term "packaged" means that the drug, compound, or composition is prepared in a manner suitable for distribution or shipping with a box, vial, pouch, bubble pack, or other protective container, which may also be used in combination. The packaging may have printing on it and/or printed material may be included in the packaging.

In preferred embodiments, the drug is selected from the drug classes or specific exemplary drugs identified in an example, in a table herein, and is subject to a regulatory limitation or suggestion or warning as described above that limits or suggests limiting approved use to patients having specific variances or variant forms of a gene identified in Examples or in the gene list provided below in order to achieve maximal benefit and avoid toxicity or other deleterious effect.

A pharmaceutical composition can be adapted to be preferentially effective in a variety of ways. In some cases, an active compound is selected which was not previously known to be differentially active, or which was not previously recognized as a potential therapeutic compound. In some cases, the concentration of an active compound which has differential activity can be adjusted such that the composition is appropriate for administration to a patient with the specified variances. For example, the presence of a specified variance may allow or require the administration of a much larger dose, which would not be practical with a previously utilized composition. Conversely, a patient may require a much lower dose, such that administration of such a dose with a prior composition would be impractical or inaccurate. Thus, the composition may be prepared in a higher or lower unit dose form, or prepared in a higher or lower concentration of the active compound or compounds. In yet other cases, the composition can include additional compounds needed to enable administration of a particular active compound in a patient with the specified variances, which was not in previous compositions, e.g., because the majority of patients did not require or benefit from the added component.

The term "differential" or "differentially" generally refers to a statistically significant different level in the specified property or effect. Preferably, the difference is also functionally significant. Thus, "differential binding or hybridization" is sufficient difference in binding or hybridization to allow

discrimination using an appropriate detection technique. Likewise, "differential effect" or "differentially active" in connection with a therapeutic treatment or drug refers to a difference in the level of the effect or activity which is distinguishable using relevant parameters and techniques for measuring the effect or activity being
5 considered. Preferably the difference in effect or activity is also sufficient to be clinically significant, such that a corresponding difference in the course of treatment or treatment outcome would be expected, at least on a statistical basis.

Also usefully provided in the present invention are probes which specifically recognize a nucleic acid sequence corresponding to a variance or variances in a gene
10 as identified in aspects above or a product expressed from the gene, and are able to distinguish a variant form of the sequence or gene or gene product from one or more other variant forms of that sequence, gene, or gene product under selective conditions. Those skilled in the art recognize and understand the identification or determination of selective conditions for particular probes or types of probes. An
15 exemplary type of probe is a nucleic acid hybridization probe, which will selectively bind under selective binding conditions to a nucleic acid sequence or a gene product corresponding to one of the genes identified for aspects above. Another type of probe is a peptide or protein, e.g., an antibody or antibody fragment which specifically or preferentially binds to a polypeptide expressed from a particular form
20 of a gene as characterized by the presence or absence of at least one variance. Thus, in another aspect, the invention concerns such probes. In the context of this invention, a "probe" is a molecule, commonly a nucleic acid, though also potentially a protein, carbohydrate, polymer, or small molecule, that is capable of binding to one variance or variant form of the gene to a greater extent than to a form of the
25 gene having a different base at one or more variance sites, such that the presence of the variance or variant form of the gene can be determined. Preferably the probe distinguishes at least one variance identified in Examples, tables or lists below or is a variance otherwise identified in a gene identified herein.

In preferred embodiments, the probe is a nucleic acid probe at least 15,
30 preferably at least 17 nucleotides in length, more preferably at least 20 or 22 or 25, preferably 500 or fewer nucleotides in length, more preferably 200 or 100 or fewer, still more preferably 50 or fewer, and most preferably 30 or fewer. In preferred embodiments, the probe has a length in a range between from any one of the above lengths to any other of the above lengths (including endpoints). In the case of
35 certain types of probes, e.g., peptide nucleic acid probes, the probe may be shorter, e.g., 6, 7, 8, 10, or 12 nucleotides in length. The probe specifically hybridizes under selective hybridization conditions to a nucleic acid sequence corresponding to a portion of one of the genes identified in connection with above aspects. The nucleic

acid sequence includes at least one variance site. Also in preferred embodiments, the probe has a detectable label, preferably a fluorescent label. A variety of other detectable labels are known to those skilled in the art. Such a nucleic acid probe can also include one or more nucleic acid analogs.

5 In preferred embodiments, the probe is an antibody or antibody fragment which specifically binds to a gene product expressed from a form of one of the above genes, where the form of the gene has at least one specific variance with a particular base at the variance site, and preferably a plurality of such variances.

10 In connection with nucleic acid probe hybridization, the term "specifically hybridizes" indicates that the probe hybridizes to a sufficiently greater degree to the target sequence than to a sequence having a mismatched base at least one variance site to allow distinguishing such hybridization. The term "specifically hybridizes" thus means that the probe hybridizes to the target sequence, and not to non-target sequences, at a level which allows ready identification of probe/target sequence
15 hybridization under selective hybridization conditions. Thus, "selective hybridization conditions" refer to conditions which allow such differential binding. Similarly, the terms "specifically binds" and "selective binding conditions" refer to such differential binding of any type of probe, e.g., antibody probes, and to the conditions which allow such differential binding. Typically hybridization reactions
20 to determine the status of variant sites in patient samples are carried out with two different probes, one specific for each of the (usually two) possible variant nucleotides. The complementary information derived from the two separate hybridization reactions is useful in corroborating the results.

Likewise, the invention provides an isolated, purified or enriched nucleic
25 acid sequence of 15 to 500 nucleotides in length, preferably 15 to 100 nucleotides in length, more preferably 15 to 50 nucleotides in length, and most preferably 15 to 30 nucleotides in length, which has a sequence which corresponds to a portion of one of the genes identified for aspects above. Preferably the lower limit for the preceding ranges is 17, 20, 22, or 25 nucleotides in length. In other embodiments, the nucleic
30 acid sequence is 30 to 300 nucleotides in length, or 45 to 200 nucleotides in length, or 45 to 100 nucleotides in length. The nucleic acid sequence includes at least one variance site. Such sequences can, for example, be amplification products of a sequence which spans or includes a variance site in a gene identified herein. Likewise, such a sequence can be a primer that is able to bind to or extend through a
35 variance site in such a gene. Yet another example is a nucleic acid hybridization probe comprised of such a sequence. In such probes, primers, and amplification products, the nucleotide sequence can contain a sequence or site corresponding to a variance site or sites, for example, a variance site identified herein. Preferably the

presence or absence of a particular variant form in the heterozygous or homozygous state is indicative of the effectiveness of a method of treatment in a patient.

In reference to nucleic acid sequences which "correspond" to a gene, the term "correspond" refers to a nucleotide sequence relationship, such that the
5 nucleotide sequence has a nucleotide sequence which is the same as the reference gene or an indicated portion thereof, or has a nucleotide sequence which is exactly complementary in normal Watson-Crick base pairing, or is an RNA equivalent of such a sequence, e.g., an mRNA, or is a cDNA derived from an mRNA of the gene.

In another aspect, the invention provides a method for determining a
10 genotype of an individual in relation to one or more variances in one or more of the genes identified in above aspects by using mass spectrometric determination of a nucleic acid sequence which is a portion of a gene identified for other aspects of this invention or a complementary sequence. Such mass spectrometric methods are known to those skilled in the art. In preferred embodiments, the method involves
15 determining the presence or absence of a variance in a gene; determining the nucleotide sequence of the nucleic acid sequence; the nucleotide sequence is 100 nucleotides or less in length, preferably 50 or less, more preferably 30 or less, and still more preferably 20 nucleotides or less. In general, such a nucleotide sequence includes at least one variance site, preferably a variance site which is informative
20 with respect to the expected response of a patient to a treatment as described for above aspects.

As indicated above, many therapeutic compounds or combinations of compounds or pharmaceutical compositions show variable efficacy and/or safety in various patients in whom the compound or compounds is administered. Thus, it is
25 beneficial to identify variances in relevant genes, e.g., genes related to the action or toxicity of the compound or compounds. Thus, in a further aspect, the invention provides a method for determining whether a compound has a differential effect due to the presence or absence of at least one variance in a gene or a variant form of a gene, where the gene is a gene identified for aspects above.

The method involves identifying a first patient or set of patients suffering
30 from a disease or condition whose response to a treatment differs from the response (to the same treatment) of a second patient or set of patients suffering from the same disease or condition, and then determining whether the occurrence or frequency of occurrence of at least one variance in at least one gene differs between the first
35 patient or set of patients and the second patient or set of patients. A correlation or other appropriate statistical test between the presence or absence of the variance or variances and the response of the patient or patients to the treatment indicates that the variance provides information about variable patient response. In general, the

method will involve identifying at least one variance in at least one gene. An alternative approach is to identify a first patient or set of patients suffering from a disease or condition and having a particular genotype, haplotype or combination of genotypes or haplotypes, and a second patient or set of patients suffering from the same disease or condition that have a genotype or haplotype or sets of genotypes or haplotypes that differ in a specific way from those of the first set of patients. Subsequently the extent and magnitude of clinical response can be compared between the first patient or set of patients and the second patient or set of patients. A correlation between the presence or absence of a variance or variances or haplotypes and the response of the patient or patients to the treatment indicates that the variance provides information about variable patient response and is useful for the present invention.

The method can utilize a variety of different informative comparisons to identify correlations. For example a plurality of pairwise comparisons of treatment response and the presence or absence of at least one variance can be performed for a plurality of patients. Likewise, the method can involve comparing the response of at least one patient homozygous for at least one variance with at least one patient homozygous for the alternative form of that variance or variances. The method can also involve comparing the response of at least one patient heterozygous for at least one variance with the response of at least one patient homozygous for the at least one variance. Preferably the heterozygous patient response is compared to both alternative homozygous forms, or the response of heterozygous patients is grouped with the response of one class of homozygous patients and said group is compared to the response of the alternative homozygous group.

Such methods can utilize either retrospective or prospective information concerning treatment response variability. Thus, in a preferred embodiment, it is previously known that patient response to the method of treatment is variable.

Also in preferred embodiments, the disease or condition is as for other aspects of this invention; for example, the treatment involves administration of a compound or pharmaceutical composition.

In preferred embodiments, the method involves a clinical trial, e.g., as described herein. Such a trial can be arranged, for example, in any of the ways described herein, e.g., in the Detailed Description.

The present invention also provides methods of treatment of a disease or condition as identified herein. Such methods combine identification of the presence or absence of particular variances, preferably in a gene or genes from Tables 1-6, 12-17, and 18-23, with the administration of a compound; identification of the presence of particular variances with selection of a method of treatment and

administration of the treatment; and identification of the presence or absence of particular variances with elimination of a method of treatment based on the variance information indicating that the treatment is likely to be ineffective or contra-
indicated, and thus selecting and administering an alternative treatment effective
5 against the disease or condition. Thus, preferred embodiments of these methods incorporate preferred embodiments of such methods as described for such sub-aspects.

As used herein, a "gene" is a sequence of DNA present in a cell that directs the expression of a "biologically active" molecule or "gene product", most
10 commonly by transcription to produce RNA and translation to produce protein. The "gene product" is most commonly a RNA molecule or protein or a RNA or protein that is subsequently modified by reacting with, or combining with, other constituents of the cell. Such modifications may include, without limitation, modification of
15 proteins to form glycoproteins, lipoproteins, and phosphoproteins, or other modifications known in the art. RNA may be modified without limitation by polyadenylation, splicing, capping or export from the nucleus or by covalent or noncovalent interactions with proteins. The term "gene product" refers to any product directly resulting from transcription of a gene. In particular this includes
20 partial, precursor, and mature transcription products (i.e., pre-mRNA and mRNA), and translation products with or without further processing including, without limitation, lipidation, phosphorylation, glycosylation, or combinations of such processing

The term "gene involved in the origin or pathogenesis of a disease or condition" refers to a gene that harbors mutations or polymorphisms that contribute
25 to the cause of disease, or variances that affect the progression of the disease or expression of specific characteristics of the disease. The term also applies to genes involved in the synthesis, accumulation, or elimination of products that are involved in the origin or pathogenesis of a disease or condition including, without limitation, proteins, lipids, carbohydrates, hormones, or small molecules.

The term "gene involved in the action of a drug" refers to any gene whose gene product affects the efficacy or safety of the drug or affects the disease process
30 being treated by the drug, and includes, without limitation, genes that encode gene products that are targets for drug action, gene products that are involved in the metabolism, activation or degradation of the drug, gene products that are involved in
35 the bioavailability or elimination of the drug to the target, gene products that affect biological pathways that, in turn, affect the action of the drug such as the synthesis or degradation of competitive substrates or allosteric effectors or rate-limiting reaction, or, alternatively, gene products that affect the pathophysiology of the

disease process via pathways related or unrelated to those altered by the presence of the drug compound. (Particular variances in the latter category of genes may be associated with patient groups in whom disease etiology is more or less susceptible to amelioration by the drug. The "action" of a drug refers to its effect on biological products within the body. The action of a drug also refers to its effects on the signs or symptoms of a disease or condition, or effects of the drug that are unrelated to the disease or condition leading to unanticipated effects on other processes. Such unanticipated processes often lead to adverse events or toxic effects. The terms "adverse event" or "toxic" event" are known in the art and include, without limitation, those listed in the FDA reference system for adverse events.

In accordance with the aspects above and the Detailed Description below, there is also described for this invention an approach for developing drugs that are explicitly indicated for, and/or for which approved use is restricted to or recommended to be restricted to individuals in the population with specific variances or combinations of variances, as determined by diagnostic tests for variances or variant forms of certain genes involved in the disease or condition or involved in the action or metabolism or transport of the drug. Such drugs may provide more effective treatment for a disease or condition in a population identified or characterized with the use of a diagnostic test for a specific variance or variant form of the gene if the gene is involved in the action of the drug or in determining a characteristic of the disease or condition. Such drugs may be developed using the diagnostic tests for specific variances or variant forms of a gene to determine the inclusion of patients in a clinical trial.

Thus, the invention also provides a method for producing a pharmaceutical composition by identifying a compound which has differential activity or effectiveness against a disease or condition in patients having at least one variance in a gene, preferably in a gene from Tables 1-6, compounding the pharmaceutical composition by combining the compound with a pharmaceutically acceptable carrier, excipient, or diluent such that the composition is preferentially effective in patients who have at least one copy of the variance or variances. In some cases, the patient has two copies of the variance or variances. In preferred embodiments, the disease or condition, gene or genes, variances, methods of administration, or method of determining the presence or absence of variances is as described for other aspects of this invention. In preferred embodiments, the active component of the pharmaceutical composition is a compound listed in the compound tables below (Tables 24 through 68), or a compound chemically related to one of the listed compounds.

Similarly, the invention provides a method for producing a pharmaceutical agent by identifying a compound which has differential activity against a disease or condition in patients having at least one copy of a form of a gene, preferably a gene from Tables 1 through 6, having at least one variance and synthesizing the compound in an amount sufficient to provide a pharmaceutical effect in a patient suffering from the disease or condition. The compound can be identified by conventional screening methods and its activity confirmed. For example, compound libraries can be screened to identify compounds which differentially bind to products of variant forms of a particular gene product, or which differentially affect expression of variant forms of the particular gene, or which differentially affect the activity of a product expressed from such gene. Alternatively, the design of a compound can exploit knowledge of the variances provided herein to avoid significant allele specific effects, in order to reduce the likelihood of significant pharmacogenetic effects during the clinical development process. Preferred embodiments are as for the preceding aspect.

In another aspect, the invention provides a method of treating a disease or condition in a patient by selecting a patient whose cells have an allele of an identified gene, preferably a gene selected from the genes listed in Tables 1 through 6. The allele contains at least one variance correlated with more effective response to a treatment of said disease or condition. The method also includes altering the level of activity in cells of the patient of a product of the allele, where the altering provides a therapeutic effect.

Preferably the allele contains a variance as shown in Tables 1-6, 12-17, and 18-23, or other variance table herein, or in Table 1 or 3 of Stanton et al., U.S. Application No. 09/300,747. Also preferably, the altering involves administering to the patient a compound preferentially active on at least one but less than all alleles of the gene.

Preferred embodiments include those as described above for other aspects of treating a disease or condition.

As recognized by those skilled in the art, all the methods of treating described herein include administration of the treatment to a patient.

In a further aspect, the invention provides a method for determining a treatment effective to treat a disease or condition by altering the level of activity of a product of an allele of a gene selected from the genes listed in Tables 1-6, and determining whether that alteration provides a differential effect (with respect to reducing or alleviating a disease or condition, or with respect to variation in toxicity or tolerance to a treatment) in patients with at least one copy of at least one allele of the gene as compared to patients with at least one copy of one alternative allele.,

The presence of such a differential effect indicates that altering the level or activity of the gene provides at least part of an effective treatment for the disease or condition.

5 Preferably the method for determining a treatment is carried out in a clinical trial, e.g., as described above and/or in the Detailed Description below.

In a further aspect, the invention provides a method for determining a treatment effective to treat a disease or condition by altering the level of activity of a product of an allele of a gene selected from the genes listed in Tables 1-6, and determining whether that alteration provides a differential effect (with respect to
10 reducing or alleviating a disease or condition, or with respect to variation in toxicity or tolerance to a treatment) in patients with at least one copy of at least one allele of the gene as compared to patients with at least one copy of one alternative allele.,
The presence of such a differential effect indicates that altering the level or activity of the gene provides at least part of an effective treatment for the disease or
15 condition.

Preferably the method for determining a method of treatment is carried out in a clinical trial, e.g., as described above and/or in the Detailed Description below.

In still another aspect, the invention provides a method for performing a clinical trial or study, which includes selecting or stratifying subjects in the trial or
20 study using a variance or variances or haplotypes from one or more genes specified in Tables 1-6, 12-17, and 18-23. Preferably the differential efficacy, tolerance, or safety of a treatment in a subset of patients who have a particular variance, variances, or haplotype in a gene or genes from Tables 1-6, 12-17, and 18-23 is determined by conducting a clinical trial and using a statistical test to assess whether
25 a relationship exists between efficacy, tolerance, or safety and the presence or absence of any of the variances or haplotype in one or more of the genes. Results of the clinical trial or study are indicative of whether a higher or lower efficacy, tolerance, or safety of the treatment in a subset of patients is associated with any of the variance or variances or haplotype in one or more of the genes. In preferred
30 embodiments, the clinical trial or study is a Phase I, II, III, or IV trial or study. Preferred embodiments include the stratifications and/or statistical analyses as described below in the Detailed Description.

In preferred embodiments, normal subjects or patients are prospectively stratified by genotype in different genotype-defined groups, including the use of
35 genotype as an enrollment criterion, using a variance, variances or haplotypes from

Tables 1-6, 12-17, and 18-23, and subsequently a biological or clinical response variable is compared between the different genotype-defined groups. In preferred embodiments, normal subjects or patients in a clinical trial or study are stratified by a biological or clinical response variable in different biologically or clinically-defined groups, and subsequently the frequency of a variance, variances or haplotypes from Tables 1-6, 12-17, and 18-23 is measured in the different biologically or clinically defined groups.

In preferred embodiments, e.g., of the above two analyses, the normal subjects or patients in a clinical trial or study are stratified by at least one demographic characteristic selected from the groups consisting of sex, age, racial origin, ethnic origin, or geographic origin.

Generally the method will involve assigning patients to a group to receive the method of treatment or to a control group.

In yet another aspect, the invention provides experimental methods for finding additional variances in a gene provided in Tables 1-6, 12-17, 18-23. A number of experimental methods can also beneficially be used to identify variances. Thus, the invention provides methods for producing cDNA (Example 1) and detecting additional variances in the genes provided in Tables 1-6, 12-17, 18-23, using the single strand conformation polymorphism (SSCP) method (Example 2), the T4 Endonuclease VII method (Example 3) or DNA sequencing (Example 4) or other methods pointed out below. The application of these methods to the identified genes will provide identification of additional variances that can affect inter-individual variation in drug or other treatment response. One skilled in the art will recognize that many methods for experimental variance detection have been described (in addition to the exemplary methods of examples 2, 3, 4) and can be utilized. These additional methods include chemical cleavage of mismatches (see, e.g., Ellis T.P., et al., Chemical cleavage of mismatch: a new look at an established method. *Human Mutation* 11(5):345-53, 1998), denaturing gradient gel electrophoresis (see, e.g., Van Orsouw N.J., et al., Design and application of 2-D DGGE-based gene mutational scanning tests. *Genet Anal.* 14(5-6):205-13, 1999) and heteroduplex analysis (see, e.g., Ganguly A., et al., Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. *Proc Natl Acad Sci U S A.* 90 (21):10325-9, 1993). Table 3 of Stanton et al., U.S. Application No. 09/300,747, provides a description of the additional possible variances that could be detected by one skilled in the art by

testing an identified gene in Tables 1-6, 12-17, 18-23, using the variance detection methods described or other methods which are known or are developed.

The present invention provides a method for treating a patient at risk for a disease, disorder, dysfunction or condition (for example to prevent or delay the onset of frank disease) or a patient already diagnosed with a said disease or a disease associated with said disease. The methods include identifying such a patient and determining the patient's genotype or haplotype for an identified gene or genes. The patient identification can, for example, be based on clinical evaluation using conventional clinical metrics and/or on evaluation of a genetic variance or variances in one or more genes, preferably a gene or genes from Tables 1-6. The invention provides a method for using the patient's genotype status to determine a treatment protocol that includes a prediction of the efficacy and/or safety of a therapy.

In another aspect, the invention provides a method for treating a patient at risk for a drug-induced disease, disorder or dysfunction by a) identifying a patient with such a risk, b) determining the genotypic allele status of the patient, and c) converting the data obtained in step b) into a treatment protocol that includes a comparison of the genotypic allele status determination with the allele frequency of a control population. This comparison allows for a statistical calculation of the patient's risk for having drug-induced disease, disorder, or dysfunction, e.g., based on correlation of the allele frequencies for a population with response or disease occurrence and/or severity. In preferred embodiments, the method provides a treatment protocol that predicts a patient being heterozygous or homozygous for an identified allele to exhibit signs and or symptoms of drug-induced disease, disorder, or dysfunction and a patient who is wild-type homozygous for the said allele, as responding favorably to these therapies.

In another related aspect, the invention provides a method for identifying a patient for participation in a clinical trial of a therapy for the treatment of a disease or an associated pathological or psychiatric condition.

The method for identification of a subject of the participation in a clinical trial of a therapy for a disease described in this invention involves determining the genotype or haplotype of a patient with (or at risk for) a disease as identified herein. Preferably the genotype is for a variance in a gene from Tables 1-6. Patients with eligible genotypes are then assigned to a treatment or placebo group, preferably by a blinded randomization procedure. In preferred embodiments, the selected patients have at least no copies, one copy or two copies of a wild type specific allele of identified a gene or genes identified in Tables 1-6. Alternatively, patients selected for the clinical trial may have zero, one or two copies of an allele belonging to a set of alleles, where the set of alleles comprise a group of related alleles. One

procedure for rigorously defining a set of alleles is by applying phylogenetic methods to the analysis of haplotypes. (See, for example: Templeton A.R., Crandall K.A. and C.F. Sing, A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III.

5 Cladogram estimation. *Genetics* 1992 Oct. 132(2):619-33.) Regardless of the specific tools used to group alleles, the trial would then test the hypothesis that a statistically significant difference in response to a treatment can be demonstrated between two groups of patients each defined by the presence of zero, one or two alleles (or allele groups) at a gene or genes. Said response may be a desired or an
10 undesired response. In a preferred embodiment, the treatment protocol involves a comparison of placebo vs. treatment response rates in two or more genotype-defined groups. For example a group with no copies of an allele may be compared to a group with two copies, or a group with no copies may be compared to a group consisting of those with one or two copies. In this manner different genetic models
15 (dominant, co-dominant, recessive) for the transmission of a treatment response trait can be tested. Alternatively, statistical methods that do not posit a specific genetic model, such as contingency tables, can be used to measure the effects of an allele on treatment response.

In another preferred embodiment, patients in a clinical trial can be grouped
20 (at the end of the trial) according to treatment response, and statistical methods can be used to compare allele (or genotype or haplotype) frequencies in two groups. For example responders can be compared to nonresponders, or patients suffering adverse events can be compared to those not experiencing such effects. Alternatively response data can be treated as a continuous variable and the ability of genotype to
25 predict response can be measured. In a preferred embodiments patients who exhibit extreme phenotypes are compared with all other patients or with a group of patients who exhibit a divergent extreme phenotype. For example if there is a continuous or semi-continuous measure of treatment response (for example the Alzheimer's Disease Assessment Scale, the Mini-Mental State Examination or the Hamilton
30 Depression Rating Scale) then the 10% of patients with the most favorable responses could be compared to the 10% with the least favorable, or the patients one standard deviation above the mean score could be compared to the remainder, or to those one standard deviation below the mean score. One useful way to select the threshold for defining a response is to examine the distribution of responses in a placebo group. If
35 the upper end of the range of placebo responses is used as a lower threshold for an 'outlier response' then the outlier response group should be almost free of placebo responders. This is a useful threshold because the inclusion of placebo responders in

a 'true' reponse group decreases the ability of statistical methods to detect a genetic difference between responders and nonresponders.
disease.

In a related aspect, the invention provides a method for developing a disease management protocol that entails diagnosing a patient with a disease or a disease
5 susceptibility, determining the genotype of the patient at a gene or genes correlated with treatment response and then selecting an optimal treatment based on the disease and the genotype (or genotypes or haplotypes). The disease management protocol may be useful in an education program for physicians, other caregivers or
10 pharmacists; may constitute part of a drug label; or may be useful in a marketing campaign.

In a related aspect, the invention provides a method for treating a patient at risk for or diagnosed with drug-induced disease or pathological condition or
dysfunction using the methods of the above aspect and conducting a step c) which
15 involves determining the gene allele load status of the patient. This method further involves converting the data obtained in steps b) and c) into a treatment protocol that includes a comparison of the allele status determinations of these steps with the allele frequency of a control population. This affords a statistical calculation of the
20 patient's risk for having drug-induced disease, disorder or dysfunction. In a preferred embodiment, the method is useful for identifying drug-induced disease, disorder or dysfunction. In addition, in related embodiments, the methods provide a treatment protocol that predicts a patient to be at high risk for drug-induced disease, disorder or dysfunction responding by exhibiting signs and symptoms of drug-induced toxicity, disorders, dysfunction if the patient is determined as having a
25 genotype or allelic difference in the identified gene or genes. Such patients are preferably given alternative therapies.

The invention also provides a method for improving the safety of candidate therapies for the identification of a drug-induced disease, disorder, or dysfunction. The method includes the step of comparing the relative safety of the candidate
30 therapeutic intervention in patients having different alleles in one or more than one of the genes listed in Tables 1-6, 12-17, and 18-23. Preferably, administration of the drug is preferentially provided to those patients with an allele type associated with increased efficacy. In a preferred embodiment, the alleles of identified gene or genes used are wild-type and those associated with altered biological activity.

35 For the aspects above, in connection with any of the listed diseases, disorders, or conditions and treatments thereof, or indeed any disease or disorder, can utilize pharmacogenetic information and determinations of genes and gene pathways involved in the absorption, distribution, metabolism, or excretion of said

treatment. Thus, the presence or the absence of at least variance or haplotype in such a gene or genes can be indicative of the effectiveness of a treatment for a given disease, disorder, or condition, where the gene or gene pathway is involved in the absorption, distribution, metabolism, or excretion of said treatment, e.g., a drug treatment.

As used herein, by "therapy associated with drug-induced disease" is meant any therapy resulting in pathophysiologic dysfunction or signs and symptoms of failure or dysfunction, or those associated with the pathophysiological manifestations of a disorder. A suitable therapy can be a pharmacological agent, drug, or therapy that alters a pathways identified to affect the molecular structure or function of the parent candidate therapeutic intervention thereby affecting drug-induced disease or disorder progression of any of the described organ system dysfunctions.

By "drug-induced disease" or "drug-induced syndrome" is meant any physiologic condition that may be correlated with medical therapy by a drug, agent, or candidate therapeutic intervention.

By "drug-induced dysfunction" is meant a physiologic disorder or syndrome that may be correlated with medical therapy by a drug, agent, or candidate therapeutic intervention in which symptomology is similar to drug-induced disease. Specifically included are: a) hemostasis dysfunction; b) cutaneous disorders; c) cardiovascular dysfunction; d) renal dysfunction; e) pulmonary dysfunction; f) hepatic dysfunction; g) systemic reactions; and h) central nervous system dysfunction.

By "drug associated disorder" is meant a physiologic dysfunction that may be correlated with medical therapy by a drug, agent, or candidate therapeutic intervention. The drug associated disorder may include disease, disorder, or dysfunction.

As used herein, by "therapy associated with inflammatory or immunological disease" is meant any therapy resulting in dysfunction or signs and symptoms of a inflammatory or immunologic condition or dysfunction, or those associated with the pathophysiological manifestations of a clinically diagnosed inflammatory or immunologic disorder or syndrome. A suitable therapy can be a pharmacological agent or drug that may enhance or inhibit metabolic pathways identified to affect the molecular structure or function of the parent candidate therapeutic intervention thereby affecting inflammatory or immunological disease progression of any of these inflammatory or immunological dysfunctions.

By "inflammatory or immunological dysfunction" is meant a disease or syndrome in which symptomology is similar to a inflammatory or immunological

disease. Specifically included are: arthritis, asthma, chronic obstructive pulmonary disease, autoimmune disease, inflammatory bowel disease, immunosuppression related to transplantation, pain associated with inflammation, psoriasis, atherosclerosis, and hepatitis.

5 By "pathway" or "gene pathway" is meant the group of biologically relevant genes involved in a pharmacodynamic or pharmacokinetic mechanism of drug, agent, or candidate therapeutic intervention. These mechanisms may further include any physiologic effect the drug or candidate therapeutic intervention renders.

10 By "disease management protocol" or "treatment protocol" is meant a means for devising a therapeutic plan for a patient using laboratory, clinical and genetic data, including the patient's diagnosis and genotype. The protocol clarifies therapeutic options and provides information about probable prognoses with different treatments. The treatment protocol may provide an estimate of the likelihood that a patient will respond positively or negatively to a therapeutic
15 intervention. The treatment protocol may also provide guidance regarding optimal drug dose and administration, and likely timing of recovery or rehabilitation. A "disease management protocol" or "treatment protocol" may also be formulated for asymptomatic and healthy subjects in order to forecast future disease risks based on laboratory, clinical and genetic variables. In this setting the protocol specifies
20 optimal preventive or prophylactic interventions, including use of compounds, changes in diet or behavior, or other measures. The treatment protocol may include the use of a computer program.

In another aspect, the invention provides a kit containing at least one probe or at least one primer (or other amplification oligonucleotide) or both (e.g., as described
25 above) corresponding to a gene or genes listed in Tables 1-6, 12-17, and 18-23 or other gene related to a disease or condition listed in Tables 7-11 or described within the invention. The kit is preferably adapted and configured to be suitable for identification of the presence or absence of a particular variance or variances, which can include or consist of a nucleic acid sequence corresponding to a portion of a gene. A plurality of variances
30 may comprise a haplotype of haplotypes. The kit may also contain a plurality of either or both of such probes and/or primers, e.g., 2, 3, 4, 5, 6, or more of such probes and/or primers. Preferably the plurality of probes and/or primers are adapted to provide detection of a plurality of different sequence variances in a gene or plurality of genes, e.g., in 2, 3, 4, 5, or more genes or to amplify and/or sequence a nucleic acid sequence
35 including at least one variance site in a gene or genes. Preferably one or more of the variance or variances to be detected are correlated with variability in a treatment response or tolerance, and are preferably indicative of an effective response to a treatment. In preferred embodiments, the kit contains components (e.g., probes and/or primers) adapted

or useful for detection of a plurality of variances (which may be in one or more genes) indicative of the effectiveness of at least one treatment, preferably of a plurality of different treatments for a particular disease or condition. It may also be desirable to provide a kit containing components adapted or useful to allow detection of a plurality of variances indicative of the effectiveness of a treatment or treatment against a plurality of diseases. The kit may also optionally contain other components, preferably other components adapted for identifying the presence of a particular variance or variances. Such additional components can, for example, independently include a buffer or buffers, e.g., amplification buffers and hybridization buffers, which may be in liquid or dry form, a DNA polymerase, e.g., a polymerase suitable for carrying out PCR (e.g., a thermostable DNA polymerase), and deoxy nucleotide triphosphates (dNTPs). Preferably a probe includes a detectable label, e.g., a fluorescent label, enzyme label, light scattering label, or other label. Preferably the kit includes a nucleic acid or polypeptide array on a solid phase substrate. The array may, for example, include a plurality of different antibodies; and/or a plurality of different nucleic acid sequences. Sites in the array can allow capture and/or detection of nucleic acid sequences or gene products corresponding to different variances in one or more different genes. Preferably the array is arranged to provide variance detection for a plurality of variances in one or more genes which correlate with the effectiveness of one or more treatments of one or more diseases, which is preferably a variance as described herein.

The kit may also optionally contain instructions for use, which can include a listing of the variances correlating with a particular treatment or treatments for a disease or diseases and/or a statement or listing of the diseases for which a particular variance or variances correlates with a treatment efficacy and/or safety.

Preferably the kit components are selected to allow detection of a variance described herein, and/or detection of a variance indicative of a treatment, e.g., administration of a drug, pointed out herein.

Additional configurations for kits of this invention will be apparent to those skilled in the art.

The invention also includes the use of such a kit to determine the genotype(s) of one or more individuals with respect to one or more variance sites in one or more genes identified herein. Such use can include providing a result or report indicating the presence and/or absence of one or more variant forms or a gene or genes which are indicative of the effectiveness of a treatment or treatments.

In another aspect, the invention provides a method for determining whether there is a genetic component to intersubject variation in a surrogate treatment response. The method involves administering the treatment to a group of related (preferably normal) subjects and a group of unrelated (preferably normal) subjects, measuring a surrogate pharmacodynamic or pharmacokinetic drug response variable in the subjects, performing a statistical test measuring the variation in response in the group of related subjects and, separately in the group of unrelated subjects, comparing the magnitude or pattern of variation in response or both between the groups to determine if the responses of the groups are different, using a predetermined statistical measure of difference. A difference in response between the groups is indicative that there is a genetic component to intersubject variation in the surrogate treatment response.

In preferred embodiments, the size of the related and unrelated groups is set in order to achieve a predetermined degree of statistical power.

In another aspect, the invention provides a method for evaluating the combined contribution of two or more variances to a surrogate drug response phenotype in subjects (preferably normal subjects) by a. genotyping a set of unrelated subjects participating in a Phase I trial of a compound. The genotyping is for two or more variances (which can be a haplotype), thereby identifying subjects with specific genotypes, where the two or more specific genotypes define two or more genotype-defined groups. A drug is administered to subjects with two or more of the specific genotypes, and a surrogate pharmacodynamic or pharmacokinetic drug response variable is measured in the subjects. A statistical test or tests is performed to measure response in the groups separately, where the statistical tests provide a measurement of variation in response with each group. The magnitude or pattern of variation in response or both is compared between the groups to determine if the groups are different using a predetermined statistical measure of difference.

In preferred embodiments, the specific genotypes are homozygous genotypes for two variances. In preferred embodiments, the comparison is between groups of subjects differing in three or more variances, e.g., 3, 4, 5, 6, or even more variances.

In another aspect, the invention provides a method for providing contract research services to clients (preferably in the pharmaceutical and biotechnology industries), by enrolling subjects (e.g., normal and/or patient subjects) in a clinical

drug trial or study unit (preferably a Phase I drug trial or study unit) for the purpose of genotyping the subjects in order to assess the contribution of genetic variation to variation in drug response, genotyping the subjects to determine the status of one or more variances in the subjects, administering a compound to the subjects and
5 measuring a surrogate drug response variable, comparing responses between two or more genotype-defined groups of subjects to determine whether there is a genetic component to the interperson variability in response to said compound; and reporting the results of the Phase I drug trial to a contracting entity. Clearly, intermediate results, e.g., response data and/or statistical analysis of response or
10 variation in response.

In preferred embodiments, at least some of the subjects have disclosed that they are related to each other and the genetic analysis includes comparison of groups of related individuals. To encourage participation of sufficient numbers of related individuals, it can be advantageous to offer or provide compensation to one or more
15 of the related individuals based on the number of subjects related to them who participate in the clinical trial, or on whether at least a minimum number of related subjects participate, e.g., at least 3, 5, 10, 20, or more.

In a related aspect, the invention provides a method for recruiting a clinical trial population for studies of the influence of genetic variation on drug response, by
20 soliciting subjects to participate in the clinical trial, obtaining consent of each of a set of subjects for participation in the clinical trial, obtaining additional related subjects for participation in the clinical trial by compensating one or more of the related subjects for participation of their related subjects at a level based on the number of related subjects participating or based on participation of at least a
25 minimum specified number of related subjects, e.g., at minimum levels as specified in the preceding aspect.

In all of the aspects herein, the gene (or genes) can be a gene as identified herein (e.g., in the Detailed Description, including examples, or Tables 1-6, 12-17, or 18-23, or is in a pathway as identified herein, e.g., in a Table.

30 By "pathway" or "gene pathway" is meant the group of biologically relevant genes involved in a pharmacodynamic or pharmacokinetic mechanism of drug, agent, or candidate therapeutic intervention. These mechanisms may further include any physiologic effect the drug or candidate therapeutic intervention renders.

Included in this are "biochemical pathways" which is used in its usual sense to refer to a series of related biochemical processes (and the corresponding genes and gene products) involved in carrying out a reaction or series of reactions. Generally in a cell, a pathway performs a significant process in the cell.

5 By "pharmacological activity" used herein is meant a biochemical or physiological effect of drugs, compounds, agents, or candidate therapeutic interventions upon administration and the mechanism of action of that effect.

The pharmacological activity is then determined by interactions of drugs, compounds, agents, or candidate therapeutic interventions, or their mechanism of action, on their target proteins or macromolecular components. By "agonist" or
10 "mimetic" or "activators" is meant a drug, agent, or compound that activate physiologic components and mimic the effects of endogenous regulatory compounds. By "antagonists", "blockers" or "inhibitors" is meant drugs, agents, or compounds that bind to physiologic components and do not mimic endogenous
15 regulatory compounds, or interfere with the action of endogenous regulatory compounds at physiologic components. These inhibitory compounds do not have intrinsic regulatory activity, but prevent the action of agonists. By "partial agonist" or "partial antagonist" is meant an agonist or antagonist, respectively, with limited or partial activity. By "negative agonist" or "inverse antagonists" is meant that a
20 drug, compound, or agent that can interact with a physiologic target protein or macromolecular component and stabilizes the protein or component such that agonist-dependent conformational changes of the component do not occur and agonist mediated mechanism of physiological action is prevented. By "modulators" or "factors" is meant a drug, agent, or compound that interacts with a target protein
25 or macromolecular component and modifies the physiological effect of an agonist.

As used herein the term "chemical class" refers to a group of compounds that share a common chemical scaffold but which differ in respect to the substituent groups linked to the scaffold. Examples of chemical classes of drugs include, for example, phenothiazines, piperidines, benzodiazepines and aminoglycosides.

30 Members of the phenothiazine class include, for example, compounds such as chlorpromazine hydrochloride, mesoridazine besylate, thioridazine hydrochloride, acetophenazine maleate trifluoperazine hydrochloride and others, all of which share a phenothiazine backbone. Members of the piperidine class include, for example,

compounds such as meperidine, diphenoxylate and loperamide, as well as phenylpiperidines such as fentanyl, sufentanil and alfentanil, all of which share the piperidine backbone. Chemical classes and their members are recognized by those skilled in the art of medicinal chemistry.

5 As used herein the term "surrogate marker" refers to a biological or clinical parameter that is measured in place of the biologically definitive or clinically most meaningful parameter. In comparison to definitive markers, surrogate markers are generally either more convenient, less expensive, provide earlier information or provide pharmacological or physiological information not directly obtainable with
10 definitive markers. Examples of surrogate biological parameters: (i) testing erythrocyte membrane acetylcholinesterase levels in subjects treated with an acetylcholinesterase inhibitor intended for use in Alzheimer's disease patients (where inhibition of brain acetylcholinesterase would be the definitive biological parameter); (ii) measuring levels of CD4 positive lymphocytes as a surrogate marker
15 for response to a treatment for acquired immune deficiency syndrome (AIDS). Examples of surrogate clinical parameters: (i) performing a psychometric test on normal subjects treated for a short period of time with a candidate Alzheimer's compound in order to determine if there is a measurable effect on cognitive function. The definitive clinical test would entail measuring cognitive function in a clinical
20 trial in Alzheimer's disease patients. (ii) Measuring blood pressure as a surrogate marker for myocardial infarction. The measurement of a surrogate marker or parameter may be an endpoint in a clinical study or clinical trial, hence "surrogate endpoint".

As used herein the term "related" when used with respect to human subjects
25 indicates that the subjects are known to share a common line of descent; that is, the subjects have a known ancestor in common. Examples of preferred related subjects include sibs (brothers and sisters), parents, grandparents, children, grandchildren, aunts, uncles, cousins, second cousins and third cousins. Subjects less closely related than third cousins are not sufficiently related to be useful as "related"
30 subjects for the methods of this invention, even if they share a known ancestor, unless some related individuals that lie between the distantly related subjects are also included. Thus, for a group of related individuals, each subject shares a known ancestor within three generations or less with at least one other subject in the group,

and preferably with all other subjects in the group or has at least that degree of consanguinity due to multiple known common ancestors. More preferably, subjects share a common ancestor within two generations or less, or otherwise have equivalent level of consanguinity. Conversely, as used herein the term "unrelated", when used in respect to human subjects, refers to subjects who do not share a known ancestor within 3 generations or less, or otherwise have known relatedness at that degree.

As used herein the term "pedigree" refers to a group of related individuals, usually comprising at least two generations, such as parents and their children, but often comprising three generations (that is, including grandparents or grandchildren as well). The relation between all the subjects in the pedigree is known and can be represented in a genealogical chart.

As used herein the term "hybridization", when used with respect to DNA fragments or polynucleotides encompasses methods including both natural polynucleotides, non-natural polynucleotides or a combination of both. Natural polynucleotides are those that are polymers of the four natural deoxynucleotides (deoxyadenosine triphosphate [dA], deoxycytosine triphosphate [dC], deoxyguanine triphosphate [dG] or deoxythymidine triphosphate [dT], usually designated simply thymidine triphosphate [T]) or polymers of the four natural ribonucleotides (adenosine triphosphate [A], cytosine triphosphate [C], guanine triphosphate [G] or uridine triphosphate [U]). Non-natural polynucleotides are made up in part or entirely of nucleotides that are not natural nucleotides; that is, they have one or more modifications. Also included among non-natural polynucleotides are molecules related to nucleic acids, such as peptide nucleic acid [PNA]). Non-natural polynucleotides may be polymers of non-natural nucleotides, polymers of natural and non-natural nucleotides (in which there is at least one non-natural nucleotide), or otherwise modified polynucleotides. Non-natural polynucleotides may be useful because their hybridization properties differ from those of natural polynucleotides. As used herein the term "complementary", when used in respect to DNA fragments, refers to the base pairing rules established by Watson and Crick: A pairs with T or U; G pairs with C. Complementary DNA fragments have sequences that, when aligned in antiparallel orientation, conform to the Watson-Crick base pairing rules at all positions or at all positions except one. As used herein, complementary DNA

fragments may be natural polynucleotides, non-natural polynucleotides, or a mixture of natural and non-natural polynucleotides.

As used herein "amplify" when used with respect to DNA refers to a family of methods for increasing the number of copies of a starting DNA fragment.

5 Amplification of DNA is often performed to simplify subsequent determination of DNA sequence, including genotyping or haplotyping. Amplification methods include the polymerase chain reaction (PCR), the ligase chain reaction (LCR) and methods using Q beta replicase, as well as transcription-based amplification systems such as the isothermal amplification procedure known as self-sustained sequence
10 replication (3SR, developed by T.R. Gingeras and colleagues), strand displacement amplification (SDA, developed by G.T. Walker and colleagues) and the rolling circle amplification method (developed by P. Lizardi and D. Ward).

As used herein "contract research services for a client" refers to a business arrangement wherein a client entity pays for services consisting in part or in whole
15 of work performed using the methods described herein. The client entity may include a commercial or non-profit organization whose primary business is in the pharmaceutical, biotechnology, diagnostics, medical device or contract research organization (CRO) sector, or any combination of those sectors. Services provided to such a client may include any of the methods described herein, particularly
20 including clinical trial services, and especially the services described in the Detailed Description relating to a Pharmacogenetic Phase I Unit. Such services are intended to allow the earliest possible assessment of the contribution of a variance or variances or haplotypes, from one or more genes, to variation in a surrogate marker in humans. The surrogate marker is generally selected to provide information on a
25 biological or clinical response, as defined above.

As used herein, "comparing the magnitude or pattern of variation in response" between two or more groups refers to the use of a statistical procedure or procedures to measure the difference between two different distributions. For example, consider two genotype-defined groups, AA and aa, each homozygous for a
30 different variance or haplotype in a gene believed likely to affect response to a drug. The subjects in each group are subjected to treatment with the drug and a treatment response is measured in each subject (for example a surrogate treatment response). One can then construct two distributions: the distribution of responses in the AA

group and the distribution of responses in the aa group. These distributions may be compared in many ways, and the significance of any difference qualified as to its significance (often expressed as a p value), using methods known to those skilled in the art. For example, one can compare the means, medians or modes of the two
5 distributions, or one can compare the variance or standard deviations of the two distributions. Or, if the form of the distributions is not known, one can use nonparametric statistical tests to test whether the distributions are different, and whether the difference is significant at a specified level (for example, the $p < 0.05$ level, meaning that, by chance, the distributions would differ to the degree measured
10 less than one in 20 similar experiments). The types of comparisons described are similar to the analysis of heritability in quantitative genetics, and would draw on standard methods from quantitative genetics to measure heritability by comparing data from related subjects.

Another type of comparison that can be usefully made is between related and
15 unrelated groups of subjects. That is, the comparison of two or more distributions is of particular interest when one distribution is drawn from a population of related subjects and the other distribution is drawn from a group of unrelated subjects, both subjected to the same treatment. (The related subjects may consist of small groups of related subjects, each compared only to their relatives.) A comparison of the
20 distribution of a drug response variable (e.g. a surrogate marker) between two such groups may provide information on whether the drug response variable is under genetic control. For example, a narrow distribution in the group(s) of related subjects (compared to the unrelated subjects) would tend to indicate that the measured variable is under genetic control (i.e. the related subjects, on account of
25 their genetic homogeneity, are more similar than the unrelated individuals). The degree to which the distribution was narrower in the related individuals (compared to the unrelated individuals) would be proportionate to the degree of genetic control. The narrowness of the distribution could be quantified by, for example, computing variance or standard deviation. In other cases the shape of the distribution may not
30 be known and nonparametric tests may be preferable. Nonparametric tests include methods for comparing medians such as the sign test, the slippage test, or the rank correlation coefficient (the nonparametric equivalent of the ordinary correlation

coefficient). Pearson's Chi square test for comparing an observed set of frequencies with an expected set of frequencies can also be useful.

In addition to and in connection with the determination and utilization of pharmacogenetic information for treatment of proliferative disorders such as cancer, information provided by and genes identified in the following patents and applications are useful: Housman, INHIBITORS OF ALTERNATIVE ALLELES OF GENES ENCODING PROTEINS VITAL FOR CELL VIABILITY OR CELL GROWTH AS A BASIS FOR CANCER THERAPEUTIC AGENTS, U.S. Patent 5,702,890, issued December 30, 1997, and Housman et al., PCT/US98/05419, entitled TARGET GENES FOR ALLELE-SPECIFIC DRUGS. Essential and conditionally essential genes identified therein can be utilized as targets for the methods and compositions described in those documents. As an example of the use of the information provided by the listed references, LOH affecting a particular target gene provides information on the effect of a particular variance or variances in that target gene. This can be extended to evaluation of the effects of combinations of variations one or more genes subject to LOH. The utilization of conditionally essential genes is described further herein. For complete description, see the respective patent and application.

The inventors have also determined that the loss of chromosomes or chromosome segments that is characteristic of cancer cells (often termed loss of heterozygosity, or LOH) has an important interaction with gene sequence variances, in determining the effect of a treatment on a patient's cancer cells. Cancer cells with LOH may have only one copy of a gene that is present in two copies in normal cells. If the two normal copies (one inherited from each parent) differ in activity in a given patient, then the cancer cells will be functionally different from the normal cells on account of having only one of the two copies. For example, consider a patient heterozygous for high and low activity forms of a gene that metabolizes a cancer drug. If LOH involving the chromosome containing the gene has left cancer cells with only one copy of the gene then the metabolism of the drug will be different in cancer cells compared to normal cells. If the gene copy that is lost by LOH is the high activity version then cancer cells may experience higher levels of drug (due to slower metabolism) than normal cells. Provided in this invention are specific chromosomal sites characterized by LOH, and the frequency of LOH in different types of neoplasia at said sites. These LOH sites, in conjunction with the variances described above, may prove more useful in predicting response to treatment than variances alone. Methods for determining the combined impact of LOH and variances are described herein.

It was recognized that environmental factors can cause certain genes to be essential that are not essential under other conditions (including usual *in vivo* and culture conditions). For example, certain genes involved in intermediary metabolism are not essential if the cell or organism is supplemented with high concentrations of a particular nutrient or chemical entity, but if that nutrient or chemical entity is absent or present at low levels, the gene product is essential. In another example, the administration of a drug that inhibits one or more functions within the cell can cause other functions to be essential that are not essential in the absence of the drug. In another example, subjecting a cell to harsh physical agents, such as radiation, can cause certain genes to be essential that are not essential under normal conditions. Such genes are essential under certain conditions associated with the therapy of cancer. The demonstration that such genes are present in the population in more than one allelic form and are subjected to loss of heterozygosity in cancer or noncancer proliferative disorders makes such genes targets for allele specific drugs for the treatment of such disorders.

It was found that such genes, similar to generally essential genes, are frequently deleted due to LOH in cells of proliferative disorders such as cancers. Treatment methods involving such genes can provide enhanced sensitivity of cancer cells to a variety of different anti-proliferative treatments, including radiation and administration of various compounds. Unless otherwise indicated, the term "essential" includes both strictly essential and beneficial to cell growth or survival.

A gene is said to be "conditionally essential" if it is essential for cell survival or proliferation in a specific environmental condition differing from usual *in vivo* conditions or usual culture conditions for the type of cell, where the specific environmental condition is caused by the presence or absence of specific environmental constituents, pharmaceutical agents, including small molecules or biologicals, or physical factors such as radiation, or if the gene enhances the growth or survival of the cell under such conditions by at least 2-fold, preferably by at least 4-fold, and more preferably by at least 6-fold, 10-fold or even more.

Cancer cells, as well as cells from a number of different non-malignant proliferative disorders, from an individual almost invariably undergo a loss of genetic material (DNA) when compared to normal cells. Frequently, this deletion of genetic material includes the loss of one of the two alleles of genes for which the normal somatic cells of the same individual are heterozygous, meaning that there are differences in the sequence of the gene on each of the parental chromosomes. The loss of one allele in the cancer cells is referred to as "loss of heterozygosity" (LOH). Recognizing that almost all, if not all, varieties of cancer undergo LOH, and that regions of DNA loss are often quite extensive, the genetic content of deleted regions

in cancer cells was evaluated and it was found that a variety of different conditionally essential genes are frequently deleted, reducing the cancer cell to only one copy. In this context, the term "deleted" refers to the loss of one of two copies of a chromosome or sub-chromosomal segment. Further investigation demonstrated that the loss of genetic material from cancer cells sometimes results in the selective loss of one of two alleles of a particular gene at a particular locus or loci on a particular chromosome.

The term "proliferative disorder" refers to various cancers and disorders characterized by abnormal growth of somatic cells leading to an abnormal mass of tissue which exhibits abnormal proliferation, and consequently, the growth of which exceeds and is uncoordinated with that of the normal tissues. The abnormal mass of cells is referred to as a "tumor", where the term tumor can include both localized cell masses and dispersed cells. The term "cancer" refers to a neoplastic growth and is synonymous with the terms "malignancy", or "malignant tumor". The treatment of cancers and the identification of anticancer agents is the concern of particularly preferred embodiments of the aspects of the present invention. Other abnormal proliferative diseases include "nonmalignant tumors", and "dysplastic" conditions including, but not limited to, leiomyomas, endometriosis, benign prostate hypertrophy, atherosclerotic plaques, and dysplastic epithelium of lung, breast, cervix, or other tissues. Drugs used in treating cancer and other non-cancer proliferative disorders commonly aim to inhibit the proliferation of cells and are commonly referred to as antiproliferative agents.

"Loss of heterozygosity", "LOH", or "allele loss" refers to the loss of one of the alleles of a gene from a cell or cell lineage previously having two alleles of that gene. Normal cells contain two copies of each gene, one inherited from each parent. When these two genes differ in their gene sequence, the cell is said to be "heterozygous". The term heterozygous indicates that a cell contains two different allelic forms of a particular gene and thus indicates that the allelic forms differ at at least one sequence variance site. When one allele is lost in a cell, that cell and its progeny cells, comprising its cell lineage, become "hemizygous" for that gene or "partially hemizygous" for a set of genes, and heterozygosity is lost. LOH occurs in all cancers and is a common characteristic of non-malignant, proliferative disorders. In general, many different genes will be affected by loss of heterozygosity in a cell which undergoes loss of heterozygosity. In many cancers 10-40% of all of the genes in the human genome (there are estimated to be 60,000-100,000 different genes in the genome) will exhibit LOH. In the context of this invention, these terms refer preferably to loss of heterozygosity of a gene that has a particular sequence variance in normal somatic cells of an individual such that there is loss of heterozygosity with

respect to that particular sequence variance. Also preferably, these terms refer to loss of heterozygosity of a particular sequence variance that is recognized by an inhibitor that will inhibit one allele of the gene present in normal cells of the individual, but not an alternative allele.

5

The present invention provides a number of advantages. For example, the methods described herein allow for use of a determination of a patient's genotype for the timely administration of the most suitable therapy for that particular patient. The methods of this invention provide a basis for successfully developing and obtaining regulatory approval for a compound even though efficacy or safety of the compound in an unstratified population is not adequate to justify approval. From the point of view of a pharmaceutical or biotechnology company, the information obtained in pharmacogenetic studies of the type described herein could be the basis of a marketing campaign for a drug. For example, a marketing campaign that emphasized the superior efficacy or safety of a compound in a genotype or haplotype restricted patient population, compared to a similar or competing compound used in an undifferentiated population of all patients with the disease. In this respect a marketing campaign could promote the use of a compound in a genetically defined subpopulation, even though the compound was not intrinsically superior to competing compounds when used in the undifferentiated population with the target disease. In fact even a compound with an inferior profile of action in the undifferentiated disease population could become superior when coupled with the appropriate pharmacogenetic test.

By "comprising" is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

First a brief description of the Tables is provided.

Tables 1-6, Gene Tables, lists genes that may be involved in
5 pharmacological response to cancer or other neoplastic disorders, neurological and
psychiatric, adsorption, distribution, metabolism, or excretion of, inflammation and
immune, endocrine and metabolism, and cardiovascular and renal therapeutics,
respectively, or that may define disease subsets with different prognosis and
consequent implications for treatment. These tables have seven columns. Column
10 1, headed "Class" provides broad groupings of genes relevant to the pharmacology
of indication-specific drugs. Column 2, headed "Pathway", provides a more detailed
categorization of the different classes of genes by indicating the overall purpose of
large groups of genes. These pathways contain genes implicated in the etiology or
treatment response of the various diseases detailed in Tables 7-11. Column 3,
15 headed "Function", further categorizes the pathways listed in column 2. Some
categories in column 2 (e.g. "Clotting") are not further categorized in column 3.

In column 4, headed "Name", lists the genes belonging to the class, pathway
and function shown to the left (in columns 1 - 3). The gene names given are
generally those used in the OMIM database or in GenBank, however one skilled in
20 the art will recognize that many genes have more than one name, and that it is a
straightforward task to identify synonymous names. For example, many alternate
gene names are provided in the OMIM record for a gene.

In column 5, headed "OMIM", the Online Mendelian Inheritance in Man
(OMIM) record number is listed for each gene in column 4. This record number
25 can be entered next to the words: "Enter one or more search keywords:" at the
OMIM world wide web site. The url is:
<http://www3.ncbi.nlm.nih.gov/Omim/searchomim.html>. An OMIM record exists for
most characterized human genes. The record often has useful information on the
chromosome location, function, alleles, and human diseases or disorders associated
30 with each gene.

In column 6, headed "GID", provides the GenBank identification number
(hence GID) of a genomic, cDNA, or partial sequence of the gene named in column
4. Usually the GID provides the record of a cDNA sequence. Many genes have
multiple Genbank accession numbers, representing different versions of a sequence
35 obtained by different research groups, or corrected or updated versions of a
sequence. As with the gene name, one skilled in the art will recognize that
alternative GenBank records related to the named record can be obtained easily. All
other GenBank records listing sequences that are alternate versions of the sequences

named in the table are equally suitable for the inventions described in this application. (One straightforward way to obtain additional GenBank records for a gene is on the internet. General instructions can be found at the NCBI web site at: <http://www3.ncbi.nlm.nih.gov>. More specifically, the GenBank record number in column 6 can be entered at the url:

<http://www3.ncbi.nlm.nih.gov/Entrez/nucleotide.html>. Once the GenBank record has been retrieved one can click on the "nucleotide neighbors" link and additional GenBank records from the same gene will be listed.

Column 7, headed "locus", provides the chromosome location of the gene listed on the same row. The chromosome location helps confirm the identity of the named gene if there is any ambiguity.

Tables 7-11 are matrix tables showing the intersection of genes and therapeutic indications – that is, which categories of genes are most likely to account for interpatient variation in response to treatments for which diseases. The first two columns provide a framework for organizing the genes listed in Tables 1-6. Column 1 is similar to the 'Class' column in Tables 1-6, while column 2 is a combination of the 'Pathway' and 'Function' columns in Tables 1-6. It is intended that the summary terms listed in columns 1 and 2 be read as referring to all the genes in the corresponding sections of Tables 1-6. The remaining columns in Tables 7-11 list the specific indications for a given disease category, for example in Table 7 there are thirteen neurological and psychiatric indications. The information in the Tables lies in the shaded boxes at the intersection of various 'Pathways' (the rows) and treatment indications. An intersection box is shaded when a row corresponding to a particular pathway (and by extension all the genes listed in that pathway in Tables 1-6) intersects a column for a specific neurological or psychiatric disease such that the pathway and genes are of possible use in explaining interpatient differences in response to treatments for the neurological or psychiatric indication. Thus, the Tables enables one skilled in the art to identify therapeutically relevant genes in patients with one of the listed indications for the purposes of stratification of these patients based upon genotype and subsequent correlation of genotype with drug response. The shaded intersections indicate preferred sets of genes for understanding the basis of interpatient variation in response to therapy of the indicated disease indication, and in that respect are exemplary. Any of the genes in the tables may account for interpatient variation in response to treatments for any of the diseases listed. Thus, the shaded boxes indicate the gene pathways that one skilled in the art would first investigate in trying to understand interpatient variation in response to therapy for the listed neurological indications.

Tables 12-17 lists the exemplary DNA sequence variances in genes for therelevant to the methods described in the present invention. These variances were discovered by the inventors in studies of selected genes listed in Tables 1-6, and are provided here as useful for the methods of the present invention. The variances in
5 Tables 12-17 were discovered by one or more of the methods described below in the Detailed Description or Examples. The tables have eight columns. The column headings are spread over two rows, with five headings in the first row and three in the second row. The gene sequence variance listings in the tables have a similar organization to the column headings, with a set of nomenclature data in the first row
10 for each gene entry, and variance data in the second and additional following rows for however many sequence variances are available for a specific gene. Column 1, the "Name" column, contains the Human Genome Organization (HUGO) identifier for the gene. Column 2, the "GID" column provides the GenBank accession number of a genomic, cDNA, or partial-sequence of a particular gene. Column 3, the
15 "OMIM_ID" column contains the record number corresponding to the Online Mendelian Inheritance in Man database for the gene provided in columns 1 and 2. This record number can be entered at the world wide web site <http://www3.ncbi.nlm.nih.gov/Omim/searchomim.html> to search the OMIM record on the gene. Column 4, the VGX_Symbol column, provides an internal identifier
20 for the gene. Column 5, the "Description" column provides a descriptive name for the gene, when available. Columns 6, 7 and 8 are on the second row of columns. Column 6, the "Variance_Start" column provides the nucleotide location of a variance with respect to the first listed nucleotide in the GenBank accession number provided in column 2. That is, the first nucleotide of the GenBank accession is
25 counted as nucleotide 1 and the variant nucleotide is numbered accordingly. Column 7, the "variance" column provides the nucleotide location of a variance with respect to an ATG codon believed to be the authentic ATG start codon of the gene, where the A of ATG is numbered as one (1) and the immediately preceding nucleotide is numbered as minus one (-1). This reading frame is important because
30 it allows the potential consequence of the variant nucleotide to be interpreted in the context of the gene anatomy (5' untranslated region, protein coding sequence, 3' untranslated region). Column 7 also provides the identity of the two variant nucleotides at the indicated position. Column 8, the "CDS_Context" column indicates whether the variance is in a coding region but silent (S); in a coding region
35 and results in an amino acid change (e.g., R347C, where the letters are one letter amino acid abbreviations and the number is the amino acid residue in the encoded amino acid sequence which is changed); in a sequence 5' to the coding region (5); or in a sequence 3' to the coding region (3). As indicated above, interpreting the

location of the variance in the gene is contingent on the correct assignment of the initial ATG of the encoded protein (the translation start site). It should be recognized that assignment of the correct ATG may occasionally be incorrect in GenBank, but that one skilled in the art will know how to carry out experiments to definitively identify the correct translation initiation codon (which is not always an ATG). In the event of any potential question concerning the proper identification of a gene or part of a gene, due for example, to an error in recording an identifier or the absence of one or more of the identifiers, the priority for use to resolve the ambiguity is GenBank accession number, OMIM identification number, HUGO identifier, common name identifier.

Tables 18-23 lists additional DNA sequence variances (in addition to those in Tables 12-17) in genes relevant to the methods of the present invention (i.e. selected genes from Tables 1-6). These variances were identified by various research groups and published in the scientific literature. The inventors realized that these variances may be useful for understanding interpatient variation in response to treatment of the diseases listed in Tables 7-11, and more generally useful for the methods of the present invention. The layout of Tables 18-23 is identical to that of Tables 12-17, and therefore the descriptions of the rows and columns in Tables 12-17 (above) pertain to Tables 18-23, as do the caveats and other remarks.

Tables 24-68 provide lists of exemplary compounds in clinical development for the various disease indications listed in Tables 7-11. The compounds listed in the tables are exemplary; that is, the methods of the invention will apply to other compounds as well. Each table has four columns. The first column is titled "Product Name", the second column is titled "Chemical Name", the third "Action" and the fourth "Indication". Under these headings are listed rows of compounds. For each compound there is a brief summary of information about the product name, its pharmacological action and potential clinical uses. The first column, "Product Name", provides the generic name and/or alphanumeric designation of the compound, as well as its trade name in some cases (in capital letters). The second column, "Chemical Name" provides the full chemical name of the compound. The listed compounds, or compounds chemically related to those listed (e.g. by modification of one or more chemical moieties of the listed compounds), are suitable for the methods of this invention. The third column, "Action", summarizes in a word or phrase an important pharmacological action of the compound, or what is currently believed to be an important pharmacological action – in most cases additional pharmacological actions are known but not listed to conserve space; alternatively, subsequent studies may reveal additional or alternative

pharmacological actions. (Sources listed in the detailed description will help clarify whether additional pharmacological actions have been discovered.) The fourth column, "Indication", provides an exemplary disease or condition for which the compound is currently being, or has already been, developed. In many cases the compound is being, has already been, or will likely be developed for other indications. Again, one skilled in the art will know how to identify additional drug development programs for these compounds. For example, a compound in development for one neurodegenerative disease is likely to be evaluated in the treatment of other neurodegenerative diseases.

10

Detailed Description

Preferred Embodiments

I. Disease Indications

A. Neurological and Psychiatric Diseases

5 The treatment of neurological and psychiatric diseases presents a challenge to physicians and other medical practitioners because the available therapeutics are only partially effective in only a fraction of patients. Further, many currently used medicines produce serious adverse effects. Therapeutic benefits and toxic side effects have to be balanced in each patient. This requires much attention to drug
10 selection, dosage adjustment and monitoring for potential adverse events on the part of care givers – effectively a new pharmacokinetic and pharmacodynamic study must be performed for each patient. These limitations of therapy are especially true of the most debilitating neurological and psychiatric diseases such as psychosis, depression, epilepticepilepsy, the neurodegenerative diseases including Alzheimer's
15 disease and Parkinson's disease, migraine and cerebrovascular disease. Although these diseases have distinct clinical presentations, havethere is extensive overlap in pathogenetic mechanisms and symptoms.

 Difficulties in treating neurological and psychiatric diseases are attributable to factors such as limited understanding of disease condition pathophysiology, lack
20 of specificity of pathophysiologic changes (i.e. variation in pathophysiologic machanisms in patients with similar clinical presentation) and lack of specificity of therapeutic compounds. Further, most medical therapy is directed to the amelioration of symptoms, not the arrest or reversal of underlying pathophysiologic processes. One good example of the difficulty of developing and marketing
25 effective treatments is the history of therapeutic candidates for Alzheimer's disease. Out of dozens of candidate treatments tested in clinical trials only two products have been approved for use in the United Statese, and one of them (tacrine; Cognex) has been withdrawn from marketing due to safety problems. Further, the one marketed product (donezepil; Aricept) is only used by a small fraction of eligible patients
30 because it has a reputation among caregivers and Alzheimer's disease advocacy groups as being ineffective in most patients. Thus a drug that enjoys a monopoly position in a major disease is not a great commercial success because its shortcomings are widely realized.

 In summary, medical management of neurological and psychiatric diseases is
35 empirical in nature, is only partially effective, and is associated with multiple undesirable side effects. In view of these clinical realities, the use of genetic tests to identify treatment responders, nonresponders, and/or those likely to develop undesirable side effects will have a major impact on use of existing classes of CNS

drugs, as well as on the development and use of new drugs to treat diseases of the central nervous system.

B. Pharmacokinetic Parameters and Effects on Efficacy

The pharmacokinetic parameters with potential effects on efficacy are
5 absorption, distribution, metabolism, and excretion. These parameters affect efficacy broadly by modulating the availability of a compound at the site(s) of action. Interpatient variation in the availability of a compound drug, agent, or candidate therapeutic intervention can result in a reduction of the available
10 compound or more compound at the site of action with a corresponding altered clinical effect. Differences in these parameters, therefore, can be a potential foundation of interpatient variability to drug response.

1. Pharmacokinetic Parameters that Result in a Reduction of Available Drug

- a. Absorption- Depending on the solubility of the drug, and its ability to
15 passively cross membranes is fundamental to the ability of the drug, agent, or candidate therapeutic intervention to effectively enter the circulation and gain access to the principle site of action. For enteral delivery or administration, absorption is a critical first step in the pharmacologic process. Within the gastrointestinal tract, absorption of a drug, agent, or
20 candidate therapeutic intervention can be affected by the pH of the contents, speed of gastric emptying, and presence of chelating or binding molecules to the drug, agent or candidate therapeutic intervention. Each of these parameters can effectively reduce the rate of passive absorption of the drug across the gastrointestinal mucosal membrane.
25
- b. Distribution- Once absorbed, the drug, agent or candidate therapeutic intervention must be delivered or distributed to the primary site of pharmacologic action. Although distribution is dependent on regional blood flow and cardiac output; distribution may be further affected by the rate and
30 extent of sequestration of the drug into biological spaces that render the product unavailable to the principle or primary site of pharmacologic site of action. For example, many drugs are actively transported into biological compartments. These processes, if over- or under active may affect the availability and hence reduce the efficacy of the product. Further, only
35 unbound drug may be effective to a cell, tissue, or physiological process, and bound product may be transported to a space that is physiologically unrelated to the pharmacologic mechanism of action or may be of deleterious adverse or toxic consequence.

- 5 c. Metabolism- Induction of metabolic enzymes to covalently modify the parent drug, agent or candidate therapeutic intervention may reduce the ability of the parent drug to elicit a pharmacologic action. Metabolism may affect the target active site binding, rate and extent of distribution and excretion, and overall availability of the active molecule.
- d. Excretion- If the excretion of the drug or drug metabolite is rapid, less drug is available to elicit a pharmacologic effect.

2. Pharmacokinetic Parameters that Result in More Available Drug.

- 10 a. Absorption- Enhanced absorption of drugs, agents or candidate therapeutic interventions may result in increased drug availability. For example, in some cases of decreased gastric emptying, there is an enhanced degree of absorption by prolonging contact with gastrointestinal mucosal membranes. In others, a change in the solubility of the drug may enhance the passive transport across the gastrointestinal mucosal membrane.
- 15 b. Distribution- Since free drug is the form that renders pharmacologic action and is metabolised and excreted, drug binding may serve to protect the drug from mechanisms of inactivation. The rate and extent of drug binding affects the free drug concentration relative to the total concentration.
- 20 c. Metabolism- If drug metabolism induction is occurring and the inducer is rapidly removed without adjustment in the dose of the drug, drug metabolism may be decreased and adverse effects or toxicities may occur.
- 25 d. Excretion- If inhibition of active transport of the parent drug or metabolite across the bile cannicula or the renal tubule, there is a net result of enhanced drug availability.

30 **C. Impaired Drug Tolerability and Drug-Induced Disease, Disorder, Dysfunction or Toxicity**

In response to chemical substances, drugs, or xenobiotics, drug-induced disease, disorder, dysfunction, or toxicity manifests as cellular damage or organ physiologic dysfunction, with one potentially leading to the other.

35

Adverse drug reactions can be categorized as 1) mechanism based reactions which are exaggerations of pharmacologic effects and 2) idiosyncratic, unpredictable effects unrelated to the primary pharmacologic action. Although some

side effects appear shortly after administration of a drug, some side effects appear long after drug administration or after cessation of the drug. Furthermore, these reactions can be categorized by reversible or irreversible manifestations of the drug-induced toxicity referring to whether the clinical symptomology subsides or persists upon withdrawal of the offending agent.

In the first category, excessive drug effects may result from alterations of pharmacokinetic parameters by either drug-drug interactions, pathophysiologic disease mediated alterations in the organs or processes involved in absorption, distribution, metabolism, or excretion, or genetic predisposition to heightened pharmacodynamic effect of the drug. The excessive or heightened response may be receptor or drug target or non-receptor or non-drug target mediated.

There are a large number of adverse events that are suspected and or known to occur as a result of administration of a drug, agent, or candidate therapeutic intervention. For example, many antineoplastic agents act by prevention of cell division in dividing cells or promoting cytotoxicity via disruption of DNA synthesis, transcription, and formation of mitotic spindles. These agents, unfortunately, do not distinguish between normal and cancerous cells, e.g. normally dividing cells and cancer cells are equally killed. Therefore, adverse events of antineoplastic agents include bone marrow suppression leading to anemia, leukopenia, and thrombocytopenia; immunosuppression rendering the patient susceptible and vulnerable to infectious agents; and initiation of mutagenesis and the formation of alternate forms of cancer, in many cases, acute myeloid leukemia.

In another example of predictable adverse events related to drug therapy is immunosuppression as a result of therapy to reduce or ablate immune response. This therapy includes but is not exclusive to prevention of graft vs. host or autoimmune disease. These agents, e.g. corticosteroids, cyclosporine, and azathioprine, all suppress humoral or cell-mediated immunity. Patients taking these agents are rendered susceptible to microbial infections, particular opportunistic infections such as cytomegalovirus, pneumocystis carinii, Candida, and sperigillus. Furthermore, long-term immunosuppressive therapy is associated with increased risk of developing lymphoma. Individual drugs are associated with renal injury (cyclosporine) and interstitial pneumonitis (azathioprine).

In the second category of adverse events, idiosyncratic reactions arise often by unpredictable, unknown mechanisms or reactions that evoke immunologic reactions or unanticipated cytotoxicity.

Adverse reactions in this category are often found together, because often it is difficult to ascertain the etiology of the offending reaction. These toxic events can be specific for a target organ, e.g. ototoxicity, nephrotoxicity, hepatotoxicity,

neurotoxicity, etc. or are caused by reactive metabolic intermediates and are toxic or create local damage usually near the site of metabolism.

Immunologic reactions to drugs are thought or result from the combination of the drug or agent with a protein to form an antigenic protein-drug complex that stimulates the immune system response. Without the formation of a complex, most small molecular drugs are unable, alone, to elicit an immunological response. First exposure to the offending drug produces a latent reaction, subsequent exposures usually results in heightened and rapid immunological response. These allergic reactions, characterized by immunohypersensitivity, are most dramatic in anaphylaxis. There are other immune responses that result in adverse reactions or toxicities they include but are not limited to : 1) immune response mediated cytotoxicity which occurs when the drug-protein complex binds to the surface of a cell and this cell-complex is then recognized by circulating antibodies; 2) serum sickness which occurs when immune complexes of drug and antibody are found in the circulation; and 3) lupus syndromes in which the drug or reactive intermediate interact with nuclear material to stimulate the formation of antinuclear antibodies.

In addition to the immune phenomena described above, there are other drug reactions that are syndromes involving allergic reactions. These reactions include, but are not limited to, skin e rashes, drug-induced fever, pulmonary reactions, heaptocellular or cholestatic reactions, interstitial nephritis, and lymphadenopathy. Further, there are some drug reactions that mimic allergic reactions but are not immune related. For example, such reactions are due to direct release of mediators by drugs and are called anaphylactoid reactions. An example of this type of adverse event is reaction to radiocontrast dye.

These are common adverse drug reactions that may prevent a candidate therapeutic intervention from use, continued development, and marketing rights. Some of these reactions are reversible, others are not.

Adverse drug reactions include, but are not limited to, the following organs systems: a) hemostasis which encompass blood dyscrasias (feature of over half of all drug-related deaths) which are bone marrow aplasia, granulocytopenia, aplastic anemia, leukopenia, pancytopenia, lymphoid hyperplasia, hemolytic anemia, and thrombocytopenia; b) cutaneous which encompass urticaria, macules, papules, angioedema, morbilliform-maculopapular rash, toxic epidermal necrolysis, erythema multiforme, erythema nodosum, contact dermatitis, vesicles, petechiae, exfoliative dermatitis, fixed drug eruptions, and severe skin rash (Stevens-Johnson syndrome); c) cardiovascular which includes arrhythmias, QT prolongation, cardiomyopathy, hypotension, or hypertension; d) renal which includes glomerulonephritis and tubular necrosis; e) pulmonary which includes asthma, acute pneumonitis,

eosinophilic pneumonitis, fibrotic and pleural reactions, and interstitial fibrosis; f) hepatic which includes steatosis, hepatocellular damage and cholestasis; g) systemic which includes anaphylaxis, vasculitis, fever, lupus erythematosus syndrome; and h) the central nervous system which includes tinnitus and dizziness, acute dystonic reactions, parkinsonian syndrome, coma, convulsions, depression and psychosis, and respiratory depression.

In the cases whereby severe, fatal reactions occur after drug administration, there may be a warning label in the product insert.

For example, tricyclic antidepressants can cause central nervous system depression, seizures, respiratory arrest, cardiac arrhythmias and arrest. The mechanism for the injury is a result of the increased synaptic concentrations of biogenic amines and inhibition of postsynaptic receptors.

Acetaminophen can cause hepatic necrosis as a result of prolonged high dose usage or overdose. In the hepatocyte, acetaminophen is converted to a toxic metabolite that binds to glutathione. As the concentration of acetaminophen increases the levels of glutathione are depleted and the toxic acetaminophen metabolite then binds liver macromolecules. Aggregation of polymorphonuclear neutrophils in hepatic microcirculation may cause ischemia and foster necrotic events.

Halothane can cause hepatic necrosis as well as prodrome fever and jaundice. Interestingly, the liver effects of halothane are usually after a first time exposure. The hepatic reaction is thought to occur via a genetic predisposition to deranged metabolism with the formation of toxic metabolites.

D. Pharmacokinetic Parameters as Potential Mechanisms of Drug-Induced Adverse Reactions Leading to Disease, Disorder, Dysfunction or Toxicities

1. Absorption

Absorption is the pharmacokinetic parameter that describes the rate and extent of the drug, agent, or candidate therapeutic intervention leaves the site of administration. Although absorption is critical for the drug, agent, or candidate therapeutic intervention to ultimately reach the site of physiologic action, the term bioavailability is the parameter that is clinically relevant. Bioavailability is the term used to define the extent to which the active component of the drug, agent, or candidate therapeutic intervention reaches the its site of physiologic action or a biological fluid to which has access to the site of biological action. Although bioavailability is related to all pharmacokinetic parameters, e.g. absorption, distribution, metabolism, and excretion, bioavailability is primarily dependent on the

first ability of the drug, agent, or candidate therapeutic intervention to be absorbed from the site of delivery, i.e. cross cellular membranes.

There are many factors that influence absorption of a drug, agent, or candidate therapeutic intervention. For example, compound solubility, conditions of absorption, and route of administration. In the present invention, we concern ourselves with genes that are involved in the active or passive process of drug, agent, or candidate therapeutic intervention absorption through a biological membrane.

The absorption surface is dependent on the route of administration. For example, absorption of drugs can occur via 1) oral (enteral); 2) sublingual; 3) injections (parenteral, i.e., intravenous, intramuscular, intraarterial, intrathecal, intraperitoneal, or subcutaneous); 4) rectal; 5) inhalation (pulmonary); 6) topical application (skin and eye). In each of these routes of administration, the adsorption rate and extent is dependent on the concentration of the drug at the site, the patency of the epithelial cells, local biological conditions, and function of the active or passive transport.

Absorption can affect both the efficacy and safety of a drug, agent, or candidate therapeutic intervention. For example, for a compound to achieve full pharmacologic potential, it must be available at the target site, be active, and be unbound. In regards to safety, absorption affects safety in one or more of the following: site of delivery pain, necrosis, or irritation; rate of administration; and erratic available concentrations.

2. Distribution

The distribution of the drug, agent, or candidate therapeutic intervention is dependent on the rate and extent the compound enters the bloodstream. Once in the bloodstream, the compound may be distributed to the interstitial and cellular fluids. The distribution of drugs to target tissues can be categorized into two phases. The first distribution phase, is dependent on cardiac output and regional blood flow, both of which are dependent on the health and status of the cardiovascular system. In a second distribution phase, diffusion into tissues is dependent on the level and extent that the drug, agent, or candidate therapeutic intervention is bound. Drug binding by proteins found in the blood can serve to protect the compound from modifications by enzymes, proteins, or compounds in the circulation and or limit the bioavailability of the compound to enter target tissues or individual cells.

Drug entry into tissues requires free drug, and drug binding proteins may limit this active or passive transport. Once distributed into tissues, the drug may be

sequestered within that tissue, to render full pharmacologic activity or to prevent that drug from reaching the appropriate target tissue.

Distribution can affect both the efficacy and safety of a drug, agent, or candidate therapeutic intervention. For example, for a compound to achieve full pharmacologic potential, it must be available at the target site, be active, and be unbound. In regards to safety, distribution affects safety in one or more of the following: distribution to a tissue that is more or less affected by the pharmacologic action of the compound, erratic available concentrations, and tissue specific distribution characteristics.

3. Metabolism

Drugs or xenobiotics, are usually found in the circulation bound to plasma proteins, generally but not exclusive to serum albumin. It is the bound form of the drug that is taken up by the hepatocyte. Bile salts in the circulation are taken up via organic anion transporters. Once inside the hepatocyte, the drug or bile salt is a substrate for a series of reactions that are either oxidative or reductive or reactions that are conjugative steps in the metabolism of the substrate. Generally these chemical modifications are a refined process to render the substrate more hydrophilic, or polar, to be more likely excreted in the bile (via the intestinal tract) or urine (via the kidneys). However, there are exceptions whereby the redox reactions produce reactive intermediates or products that retard elimination. Except for their role in detoxification, there is little in common among the enzymes involved in the redox detoxification reactions. For certain enzymes there are specific groups that will act as substrates, for others there are general classes of chemical compounds that will be suitable substrates for a given enzyme or enzymes.

In the mammalian liver these mechanisms to detoxify and/or enhance the excretion of metabolic by-products, endogenous substrates, and exogenous molecules. The ability to determine whether hepatic function is inadequate is based upon clinical observation, e.g., the presence of jaundice, right upper quadrant abdominal discomfort or pain, pruritis, or by clinical laboratory analyses, e.g., aspartate transaminase (AST or SGOT) or alanine transferase (ALT or SGPT). The hepatic metabolic and excretory mechanisms are critical for short- and long-term survival and are inheritable characteristics. These hepatic biotransformations mechanisms have broad substrate specificity that have been evolutionarily inherited for the host protection from environmental, biological, and chemical substances.

There are two categories of drug, agent, or candidate therapeutic intervention biotransformation (metabolism). In the first, phase I, functionalization reactions occur. Phase I reactions introduce or expose a functional group to the parent

compound. In general, phase I reactions render the parent compound pharmacologically inactive, however there are examples of phase I reaction activation or retention of activity. In phase II reactions, biosynthetic reactions occur. Phase II conjugation reactions leads to a covalent linkage between a
5 functional group on the parent compound with glucuronic acid, sulfate, glutathione, amino acids, or acetate. The metabolic conversion of drugs is the liver, however, all tissues have enzymatic activity.

Factors affecting drug biotransformation are 1) induction of metabolizing enzymes, 2) inhibition of enzymatic reactions, and 3) genetic polymorphisms. It is
10 the interplay of these factors and the health and well being of the patient or subject that determines the fate of parent drug molecules in the body.

The first factor affecting drug biotransformation is induction of metabolizing enzyme activity. The metabolic processes that modify drugs or chemicals (oxidation, reduction, or conjugation) can be induced to significant enzymatic
15 activity. Under physiological conditions, the induction process is in place to coordinately metabolize excess substrates. The induction process can be both at the level of enzymatic activity and increased protein levels of the pertinent enzyme or enzymes. Induction may include one or several of the enzymatic pathways or processes in response to the presence of drugs, xenobiotics, endogenous substrates,
20 or metabolic by-products. There may or may not be increased toxicity as a result of increased concentrations of metabolites. Further, induction of phase I reactive processes (oxidation or reduction reactions) may or may not induce the phase II reactive processes (conjugation reactions).

The second factor affecting drug biotransformation is the inhibition of
25 metabolic enzymes. Enzymatic inhibition can occur via 1) competition of two or more substrates for the enzymatic active site, 2) suicide inhibitors, or 3) depletion of required cofactors for the enzymatic pathways or processes in phase I or phase II reactions.

In competitive inhibition, two or more drugs, xenobiotics, or substrates
30 present can interact with the active site of the enzyme. If one drug binds specifically to the enzymatic active site or to an other intracellular regulatory protein molecule, other compounds are blocked from binding and remain unbound. In this case, unmetabolized parent drug or xenobiotic remains in the circulation, potentially for extended periods of time. Competitive inhibition is dependent on the relative
35 specificity of the substrates for the enzymatic active site and the concentration of the drugs or substrates. An example of competitive drug biotransformation inhibition are cimetidine and ketoconazole which inhibit oxidative drug metabolism by forming a tight complex with the heme iron complex of cytochrome P450, and

macrolide antibiotics such as erythromycin and troleandomycin are metabolized to products bind to heme groups on the cytochrome P450 molecules.

In the second case, the inhibition of enzymes involved in the drug biotransformation process may also occur by suicide inactivation. In these cases, the drug or xenobiotic may interact and covalently modify or render inactive the enzyme involved in the metabolic pathway. In this way, the parent drug compound or molecule is not metabolized, nor is it free to interact with another molecule. Examples of suicide inactivators are secobarbital and synthetic steroids (norethindrone or ethinyl estradiol) which bind to cytochrome P450 and destroy the heme portion of the enzyme unit.

In the third case, inhibition of the enzymes involved in the drug biotransformation pathway can also occur by agents or compounds or physiological status that deplete NADPH or other cofactors required for the enzymatic reactions to occur. In the cases of phase I oxidation or reduction, lack of oxygen or NADPH, may reduce the efficiency and activity of a particular enzyme. In phase II reactions, cofactors provide specific groups for the enzymatic covalent modification of the drug or xenobiotic. These phase II-cofactors are required for conjugation biotransformation reactions to occur and depletion of these cofactors would be rate limiting.

The third factor that can affect drug biotransformation is genetic polymorphism. Differences among individuals to metabolize drugs have long been known. Observed phenotypic differences, as determined by amount of drug excreted, through polymorphically controlled pathway/s has lead to a generalized classification of slow (poor) metabolizers and fast (rapid or extensive) metabolizers. In general, poor metabolizers are those with impaired metabolism of a drug via a polymorphic pathway have been associated with an increased incidence of adverse effects. In addition, to date all major deficiencies in drug metabolizing activity are inherited as autosomal recessive traits. Fast or rapid metabolizers are those individuals with processes that extensively metabolize a drug via a polymorphic pathway. The fast or rapid metabolizers have been associated with an increased incidence of ineffective treatment. In these individuals active drug is rapidly metabolized to less active or inactive metabolites such that a reassessment of the pharmacokinetic parameters and dosing regimen may require analysis or readjustment, respectively, for appropriate therapy to occur.

The first observed and catalogued genetic polymorphism associated with drug metabolism was described for isoniazid. Isoniazid is a primary drug prescribed for the chemotherapy of tuberculosis. Marked interindividual variation in the elimination of this drug was observed and genetic studies of families revealed that

this variation was genetically controlled. Isoniazid is predominantly metabolized via N-acetylation. In the analysis of the phenotypically distinct individuals, it was shown that slow acetylators were homozygous for a recessive gene and fast acetylators were homozygous or heterozygous for the wild type gene. It has been
5 determined that the incidence of the slow acetylator phenotype is approximately 50% for U.S. caucasians and blacks, 60-70% of Northern Europeans, and 5-10% in Asians. Other drugs have been shown to be polymorphically acetylated, e.g. sulfonamides (sulfadiazine, sulfamethazine, sulfapyridine, sulfameridine, and sulfadoxine), aminoglutethimide, amonafide, amrinone, dapson, dipyron, endralazine, hydralazine, prizidilol, and procainamide. Other drugs that first
10 undergo metabolism and then polymorphically acetylated are clonazepam and caffeine.

Another common genetic polymorphism associated with oxidative metabolism is exemplified by the drug-debrisoquine (a sympatholytic
15 antihypertensive). It was discovered that variable inter-patient hypotensive response was due to differing metabolic rates of debrisoquine 4-hydroxylase. Further analysis of family studies revealed that oxidative metabolic reactions are under monogenic control. A cytochrome P450 enzyme, CYP2D6, was determined to be the target gene for debrisoquine 4-hydroxylase activity. Poor metabolizers of debrisoquine
20 are homozygous for a recessive CYP2D6 allele and rapid or fast metabolizers are homozygous or heterozygous for the wild type CYP2D6 allele. Urinary metabolic ratio can be determined after administration of a probe drug and phenotypic assignments (poor or extensive metabolizer) can be identified. The extent of debrisoquine metabolic analysis achieved clinical importance as it was determined
25 that other drugs were poorly metabolized in individuals that poorly metabolized debrisoquine. For example, anti-arrhythmics such as flecainide, propafenone, and mexiletine; antidepressants such as amitriptyline, clomipramine, desipramine, fluoxetine, imipramine, maprotiline, mianserin, paroxetine, and nortriptyline; neuroleptics such as haloperidol, perphenazine, and thioridazine; antianginals such
30 as perhexilene; opioids such as dextromethorphan and codeine; and amphetamines such as methylenedioxymethamphetamine. Further, many β -adrenergic antagonists are metabolized and are subject to polymorphic influence in elimination patterns.

Another example of a genetic polymorphism affecting oxidative metabolism was described for mephenytoin, a drug prescribed for epilepsy. It was shown that a
35 deficiency in the 4'-hydroxylation of S-mephenytoin is inherited as an autosomal recessive trait. The other main metabolic pathway, N-methylation of R-mephenytoin to 5-phenyl-5-ethylhydantoin remains unaffected. Individuals with poor metabolic rate of mephenytoin are subject to adverse central effects, i.e.

sedation. Other drugs can be grouped into the poor mephenytoin metabolizers are mephobarbital, hexobarbital, side-chain oxidization of propranolol, the demethylation of imipramine, and the metabolism of diazepam and desmethyldiazepam. Further analysis of other drugs such as the metabolism of antidepressant drugs (citalopram),
5 the proton pump inhibitor omeprazol, the antimalarial drugs pantoprazole and lansoprazole cosegregate with mephenytoin metabolites.

Because the majority of metabolic enzymes for the conduct of drug biotransformation occurs in the liver, impairment of liver function as a result of hepatic pathological conditions or other disease states can lead to alterations of
10 hepatic or other organ metabolic drug biotransformation. Liver disease pathologies such as hepatitis, alcoholic liver disease, fatty liver disease, biliary cirrhosis, and hepatocarcinomas can impair function of normal physiological metabolic pathways. Further, decreases in hepatic circulation as a result of cardiac insufficiency, hypertension, vascular obstruction, or vascular insult can affect the rate and extent of
15 drug biotransformation. For example drugs with a high hepatocyte extraction ratio would have different metabolism rates affected by alterations of hepatic circulation. Changes in liver blood flow can affect the rate and extent of the metabolism and the clearance of the parent drug. In all cases of hepatic pathological conditions, the affect on drug biotransformation and clearance of parent drugs or metabolized
20 products will be dependent on the severity and extent of the liver organ and hepatocellular damage.

Although hepatic damage may affect the metabolism and clearance of a parent drug or metabolic by-product, residual concentrations of parent drug or metabolic by-products may be deleterious to the liver and its metabolic functions.
25 Following nonparenteral (enteral) administration of a drug, a significant portion of the drug will be metabolized by intestinal or hepatic enzymes before it reaches the general circulation. This first pass effect may generate active drug (administered drug was a prodrug), inactive drug, or toxic drug. Prior to circulation of the metabolized product, circulation to the kidney, the major organ for excretion of the hydrophilic moiety, and excretion via the urine will occur. Therefore, a metabolic
30 product of hepatic metabolic pathways can affect the liver, kidney, and other organs of the body prior to excretion.

a. Phase I Drug Biotransformation: Oxidation and Reduction Reactions
35 *Enzymatic Oxidation of Drugs*

In oxidative metabolism, oxidases catalyze the transfer of electrons from substrate to oxygen, generating either hydrogen peroxide or superoxide anions. There are two oxidases present in hepatocytes; they are aldehyde oxidases and

monoamine oxidases. Both of these enzymes have broad substrate specificity and contribute broadly to the metabolism of drugs. A third oxidase, xanthine oxidase, may contribute to the oxidation of drugs, due its ability to catalyze the oxidation of heterocyclic aromatic amines, for example methotrexate and 6-mercaptopurine.

5 Xanthine oxidase in intact tissues is present as a NAD-dependent dehydrogenase, and is converted to an oxidase when there is disruption of the tissue, for example during hepatic cellular damage.

Aldehyde oxidase catalyzes the oxidation of fatty aldehydes to carboxylic acids and the hydroxylation of substituted pyridines, pyrimidines, purines, and pteridines. Generally, xenobiotic aromatic nitrogen heterocycles are metabolized by this enzyme.

Monoamine oxidase is present in two forms, A and B. They are dimeric proteins consisting of identical subunits and FAD is covalently linked to the protein through a cysteinyl residue. Catalytic cycles of monoamine oxidases A or B occur

15 in discrete steps that take an amine and convert it to an aldehyde, while in the process creating hydrogen peroxide and ammonia. These oxidases have a broad specificity; they protect mitochondrial proteins from xenobiotic amines and hydrazines. Further neurotransmitters are metabolized through this route, e.g. serotonin, dopamine, and catecholamines. Primary alkylamines containing unsubstituted methylene group or groups adjacent to the nitrogen exhibits activity. Activity increases as the length of a side chain, with optimal side length being C6. These enzymes also catalyze the oxidation of secondary and tertiary amines and acyclic amines. Hydrazines can be oxidized by these oxidases. Substrates for monoamine oxidases include but are not exclusive to the following amines:

25 benzylamine, dopamine, tyramine, epinephrine, N-methylbenzylamine, and N,N-dimethylbenzylamine; and the following hydrazines: procarbazine 1,2-dimethylhydrazine.

Mono-oxygenases are present in liver cell homogenates and contain two distinct types of xenobiotic mono-oxygenases. They are the cytochrome P450 and the flavin-dependent mono-oxygenases.

30 The liver microsomal P-450 system consists of a flavoprotein, and a family of related, but distinct, hemoproteins. The flavoprotein catalyzes the transfer of the electrons from NADPH to the hemoprotein, and is the mono-oxygenase. The reaction also requires phosphatidylcholine. The reductase is a monomeric flavoprotein that contains both FAD and FMN. The reductase is specific for NADPH as a reductant, but other oxidants can be substituted. In addition to cytochrome P-450, the flavoprotein catalyzes reduction of quinones, nitro, and azo compounds.

There are many P450 gene families. Subsequent cloning and sequence determination has afforded the ability to divide this gene family into three main groups, CYP1, CYP2, and CYP3, that are responsible for the majority of drug biotransformation. There are further subdivisions in each of these families, examples being CYP2D6, CYP3A4, CYP2E1, as well as others.

Examples of enzymatic inductive processes that affect biotransformation reactions involve the P450 gene family. Specifically, glucocorticoids and anticonvulsants induce CYP3A4; isoniazid, acetone, and chronic ethanol consumption for CYP2E1. Many inducers of the cytochrome P450 enzymes also induce conjugation metabolic enzymes, e.g. glucuronosyltransferases.

In contrast to the monooxygenases, multiple forms of the terminal oxidase (P-450) are present in the hepatocyte. There are many distinct isoforms characterized in different species including humans. It should be noted that mitochondrial P-450 exhibit little or no activity in the metabolism of drugs, xenobiotics, biological compounds, or chemicals. Representative functional groups oxidated by the microsomal P-450 system are as follows: alkanes (hexane, decane, hexadecane); alkenes (vinyl chloride, aflatoxin-B1, dieldrin); aromatic hydrocarbons (naphthylene, bromobenzene, benzo(a)pyrene, biphenyl); aliphatic amines (aminopyrine, benzphetamine, ethylmorphine); heterocyclic amines (3-acetylpyridine, 4,4'-bipyridine, quinoline); amides (N-acetylaminofluorene, urethane); ethers (indemethacin, pheancetin, p-nitroanisole); and sulfides (chloropromazine, thioanisole).

There are many P450s that have been identified in human liver. Substrate specificities vary among these P-450 dependent mono-oxygenases. For example, P4501A1 prefers polycyclic aromatic hydrocarbons; P-4501A2 prefers arylamines, arylamides; P-450A26 prefers coumarin, 7-ethoxycoumarin; P-450 2C8, 2C9, 2C10 prefers tolbutamide, hexobarbital; P-450 2C18 prefers mephenytoin; P-450 mp-1, mp-2 prefers debrisoquine and related amines; P450 2E1 prefers ethanol, N-nitrosoalkylamines, vinyl monomers; P-450 3A3, 3A4, 3A5, 3A7 prefers dihydropyridines, cyclosporin, lovastatin, aflatoxins.

The effect of genetic polymorphism of the P450s has been known for some time. For example, debrisoquine and related drugs; alfentanil, tolbutamide; (S)mephenytoin. Because the P450s can be induced by xenobiotics, an enhanced metabolic rate or efficiency can lead to one drug affecting the potency, efficacy, dosing of another. For example, women taking rifampicin or barbiturates can lead to metabolic inactivation of synthetic oral contraceptives.

The flavin-containing mono-oxygenases are the principle enzymes catalyzing the N-oxidation of tertiary amine drugs to N-oxides. The N-oxides are found in

abundance in serum. Although isoforms have been identified and the catalytic cycle is similar to the cytochrome P450 system, flavin-containing mono-oxygenases substrate specificity differs. Unlike the other flavin-bearing mono-oxygenases, these flavin-containing mono-oxygenases are present in the cell as very reactive oxygen-activated form. It is believed that particular protein structure stabilizes the nucleophilic molecule. Since the molecule is so highly reactive, precise substrate-to-enzyme fit is unnecessary. The following lists substrate types and examples oxidized by the flavin-containing mono-oxygenases: tertiary amines (trifluoperazine, bromopheniramine, morphine, nicotine, pargyline); secondary amines (desipramine, methamphetamine, propanolol); hydrazines (1,1-demethylhydrazine, N-aminopiperidine, 1-methyl-1-phenylhydrazine); thiols and disulfides (dithiothreitol, β -mercaptomethanol, thiophenol); thiocarbamides (thiourea, methimazole, propylthiouracil); sulfides (dimethylsulfide, sulindac sulfide).

Examples of drugs that undergo oxidative reactions are: N-dealkylation (imipramine, diazepam, codeine, erythromycin, morphine, tamoxifen, theophylline); O-dealkylation (codeine, indomethacin, dextromethorphan); aliphatic hydroxylation (tolbutamide, ibuprofen, pentobarbital, meprobamate, cyclosporin, midazolam); aromatic hydroxylation (phenytoin, phenobarbital, propanolol, phenylbutazone, ethinyl estradiol); N-oxidation (chlorpheniramine, dapsone); S-oxidation (cimetidine, chlorpromazine, thioridazine); deamination (diazepam, amphetamine).

b. Enzymatic Reduction of Drugs

The reductases are a class of enzymes that are involved in the metabolic reduction of xenobiotics. This class of enzymes includes the aldehyde and ketone reductases, the quinone reductases, the nitro and nitroso reductases, the azoreductases, the N-oxide reductases, and the sulfoxide reductases. These classes of enzymes are involved in sequential one-electron reduction of some functional groups and produce radicals that can produce damage cellular components directly or indirectly.

The dehydrogenases consist of alcohol dehydrogenases, aldehyde dehydrogenases, or dihydrodiol dehydrogenases. This class of enzymes is involved in the catalysis of hydrogen transfer to a hydrogen acceptor, usually a pyridine nucleotide.

c. Hydrolysis of Drugs

Alternative reactions of detoxification and metabolism of drugs and xenobiotics are initial steps of hydrolysis. Esters, amides, imides, or other

functional groups that are generated as a result of a hydrolysis reaction can alter the hydrophilicity of a molecule and enhance urinary excretion. Hydrolysis occurs both enzymatically and nonenzymatically. Hydrolysis of proteins before they are degraded has been suggested as a step in the process of the aging of intracellular proteins. Antibodies with an affinity for certain esters and certain proteases e.g. 3-phosphoglyceraldehyde dehydrogenase and carbonic anhydrase, have been shown to have esterase activity.

Enzymatic hydrolysis of drugs and xenobiotics include the following enzymes: esterases, amidases, imidases, and epoxide hydratases. Examples of drugs undergoing hydrolysis reactions are: procaine, aspirin, clofibrate, lidocaine, procainamide, indomethacin.

Other hydrolytic processes include reactions owing to both enzymes in tissues, circulation, and those elaborated by microorganisms in the lower bowel; for example, sulfatases, glucuronidases, and phosphatases.

b. Phase II Drug Biotransformation: Conjugation Reactions

In addition, to the redox reactions of the hepatocyte to detoxify or metabolize xenobiotics, there are a series of conjugation reactions. The substrates for these reactions are generally the products from the redox reactions described above.

These conjugation reactions involve donation of a suitable hydrophilic molecular group to an accepting xenobiotic or its metabolite. The major function of these covalent modifications is to render the parent compound pharmacologically inactive. The covalent addition of such a group to a parent drug or compound not only inactivates the substrate but also renders the recipient molecule more polar and is more readily excreted via the bile ducts into the intestinal tract or via the urine.

Lipophilic compounds that have one of the functional groups that can serve as an acceptor undergo enzymatic catalysis with a second, donor substrate. The conjugation reactions include the following broad categories: glucuronidation, sulfation, methylation, N-acetylation, and conjugation with amino acids. The enzymes involved in these reactions are as follows: UDP-glucuronyltransferase, alcohol sulfotransferase, amine N-sulfotransferase, phenol sulfotransferase, glutathione transferase, catechol O-methyltransferase, amine N-methyltransferase, histamine N-methyltransferase, thiol S-methyltransferase, benzoyl-CoA glycine acyltransferase, acetyltransacetylase, cysteine S-conjugate N-acetyltransferase, cysteine S-conjugate N-acetyltransferase, cysteine conjugate β -lyase, thioltransferase, and rhodanese. Each of these enzymes has donor and acceptor specificities. The importance of these reactions in the detoxification and metabolism of drugs and xenobiotics are discussed in the examples

Examples of drugs that are known to be conjugated are: glucuronidation (acetaminophen, morphine, diazepam); sulfation (acetaminophen, steroids, methyldopa); acetylation (sulfonamides, isoniazid, dapsone, clonazepam).

5 4. Excretion

Excretion of parent drugs and metabolites can occur in the excretory organs, namely the kidneys, liver, lungs, skin, and breasts (milk). The kidneys are the most important organs for the excretion of drugs and metabolites. Renal excretion involves glomerular filtration, active tubular absorption, and passive tubule
10 reabsorption. The more hydrophilic the compound is the more readily excreted via urine. In addition, many drugs and metabolites are excreted via the bile into the intestinal tract. These metabolites may be excreted in the feces, or may be reabsorbed by the gastrointestinal epithelial cell lining. Organic anions and cations, steroids, fatty acids, and other drugs may be specifically transported into the bile
15 canniculus.

In all of the metabolism and excretion routes, the physiologic goal is to detoxify and rid the body of drugs, xenobiotics, endogenous or exogenous chemicals, or compounds that may or may not be deleterious to the major organs of the body. In principle the detoxification mechanisms function to attain this goal,
20 however there are many cases of major organ toxicity upon exposure to drugs or metabolites of drugs. Although drugs and drug metabolites predominantly affect the liver and kidneys due to the circulatory and physiological processes, other organs can be affected. In the present invention, we address specific genes that may have polymorphic sites affecting metabolic rates to ultimately affect these major organ
25 functions.

a. Excretion of Drugs and Drug Metabolites via the Bile

After parent drugs or xenobiotics are metabolized by redox and or conjugation reactions, the modified products can then be actively transported into
30 the bile cannicula. The transport occurs in an energy dependent fashion requiring ATP. It has been shown that the transporters involved in the active transport from the basolateral (sinusoidal) to the apical (canalicular) surfaces of hepatocytes are members of the ATP binding cassette (ABC) family. The transmembrane electrical potential required to maintain the chemical and electrical potentials required for this
35 active transport is provided by the Na⁺/K⁺ ATPases located on the basolateral membrane. Other ion transporters are the potassium channel, sodium-bicarbonate symporter, chloride-bicarbonate anion exchanger, and the chloride channel. In the cholangiocyte there are other ion transporters, for example chloride-bicarbonate

anion exchanger, isoform 2, and other organic-solute transporters. Bile acids, phosphatidyl choline, organic anions, organic cations, and cholesterol are actively transported. Approximately 5% of the transporters is multi-drug resistance protein 1 (MDR1) and the remaining are the phospholipid transporter multi-drug resistance protein 3 (MDR3), alicular multispecific organic- anion transporter (multi-drug resistance associated protein (MRP2 or cMOAT), canalicular bile-salt-export pump (BSEP or SPGP(sister of p-glycoprotein)), sodium-taurocholate cotransporter, organic anion-transporting polypeptide, glutathione transporter, and a chloride-bicarbonate anion exchanger are also involved in the transport.

These transporters have been identified to move specific molecules or compounds across biological membranes. For example, the MDR1 protein mediates the canicular excretion of bulky lipophilic cations, e.g. anticancer drugs, calcium channel blockers, cyclosporine A, and various other drugs. In contrast, the MDR3 protein transports phosphatidyl choline from the inner-leaflet to the outer leaflet of the canicular membrane. Phosphatidyl choline then can be selectively extracted by intracanalicular bile salts and secreted into bile as vesicles or mixed micelles. MRP2 is involved in the transport of amphipathic anionic substrates e.g. leukotriene C4, glutathione-S conjugates, glucuronides (bilirubin diglucuronide and estradiol-17 β -glucuronide), sulfate conjugates, and is responsible for the generation of bile flow independent of bile salts within the bile cannicula. SPGP is the canicular bile salt export pump in the mammalian liver.

The hepatocyte has the ability to recruit the ATP-requiring transporters when faced with excessive metabolites. After synthesis, these transporters are stored in compartments that, in response to cAMP, can be actively moved through the cell to the membrane and fused to the cannicula. The active movement from the intracellular compartment to the membrane requires microtubules, cytoplasmic kinesin, cytoplasmic dynein, and calcium. It has been shown that peptides activate phosphoinositide 3 kinase, and increased turnover of phosphoinositides drives the formation of 3'phosphoinositol, which can activate the transporter in the membrane and ultimately increases movement to the cannicular membrane. Signaling pathways via the activation of rab5 stimulate the active movement of the transporters to the internal compartment.

b. Excretion of Drugs and Drug Metabolites via the Kidney

Excretion of drugs or drug metabolites via the kidney and into the urine involves three processes: 1) glomerular filtration, 2) active tubular secretion, and 3) passive tubular reabsorption. The amount of drug or metabolites entering the tubular lumen is dependent on its fractional plasma protein binding and glomerular filtration

rate. In the proximal renal tubule anions and cations are actively transported by carrier mediated tubular secretion and bases are transported by a separate system that secretes choline, histamine, and other endogenous bases. In the proximal and distal tubules there is passive reabsorption of these molecules. The concentration
5 gradient for back-diffusion is created by sodium and other inorganic ions and water.

E. Inflammatory or Immunological Disease, Disorders, or Dysfunctions

Inflammatory or immunological diseases and clinical symptoms includes diseases and processes such as: arthritis (including rheumatoid arthritis,
10 osteoarthritis, and other degenerative syndromes of the joints), asthma, chronic obstructive pulmonary disease (including bronchitis, bronchiectasis, emphysema and other pulmonary diseases associated with obstruction to air flow), interstitial or restrictive lung diseases, autoimmune disease (including systemic lupus erythematosus, scleroderma and other diseases characterized by autoantibodies),
15 transplantation (often treated with long term immunosuppressive therapy), pain associated with inflammation, psoriasis and other inflammatory skin diseases, atherosclerosis (for which there is strong data supporting the role of inflammatory pathogenetic mechanisms), and hepatitis, among other diseases. One skilled in the art will recognize that there may be overlap between some of the conditions listed.

20 Challenges in treating diseases with a significant inflammatory or immunological component include: (i) limited understanding of the pathophysiologic basis of these diseases and conditions, , (ii) a complex mix of immune/inflammatory mediators operating simultaneously, with the primary (initiating) events often unclear and the relative importance of different mediators unknown, (iii) medical interventions that rarely produce specific effects, or
25 address the underlying pathophysiologic basis of the disease or condition. Thus, medical management of inflammatory or immunologic disorders is empirical in nature, is associated with multiple undesirable side effects, and disease progression is common. Based upon these clinical realities and the difficulties drug developers face in developing new treatments for diseases with an inflammatory or
30 immunologic component, the use of genotypebased stratification to identify populations enriched for responders, nonresponders, and/or those likely to develop undesirable side effects will provide clear commercial and medical benefits. Ultimately medical practitioners and patients will also benefit from an enlarged
35 choice of medicines with superior safety and efficacy when used in conjunction with genetic diagnostic tests.

Inflammation is a complex process that comprises different cellular and physiologic events that can be initiated by tissue injury, by abnormal immune

function, or by a wide variety of other endogenous or exogenous factors, not all of which are understood. The inflammatory process can also escape normal regulatory control and become part of the disease process.

Autoimmunity is one aspect of some diseases associated with abnormal immunologic function. Such diseases are characterized by the presence of autoantibodies and oligoclonal B cell populations. Immunological reactions associated with loss of self tolerance may be localized to a specific tissue, or may be systemic. Ultimately, in severe cases, the immune system produces life threatening damage to tissues, physiological function is compromised. Autoimmunity can be initiated by a variety of endogenous (genetic predisposition and others) and exogenous (chemicals, drugs, microorganisms, and others) factors.

F. Endocrine and Metabolic Diseases

The treatment of endocrine and metabolic diseases presents a challenge to physicians and other medical practitioners because the available therapeutics are only partially effective in only a fraction of patients (e.g. antiobesity medications). Further, many currently used medicines produce serious adverse effects (e.g. long term corticosteroid therapy). Therapeutic benefits and toxic side effects have to be balanced in each patient. This requires much attention to drug selection, dosage adjustment and monitoring for potential adverse events on the part of patients and care givers. These limitations of therapy are especially true for the most debilitating endocrine and metabolic diseases such as diabetes and obesity

Difficulties in treating endocrine and metabolic diseases are attributable to factors such as limited understanding of disease pathophysiology, lack of specificity of pathophysiologic changes (e.g. different pathophysiologic mechanisms in patients with similar clinical presentation) and lack of specificity of therapeutic compounds. Further, most medical therapy is directed to the amelioration of symptoms or other secondary changes (e.g. achieving effective control of blood sugar), not the arrest or reversal of underlying pathophysiologic processes. One good example of the difficulty of developing and marketing effective treatments for metabolic and endocrine diseases is the recent history of obesity therapeutics. Only a few products have been approved for treatment of obesity in the United States, and one of them, the anorectic agent dexfenfluramine (d-FF; Redux), a 5-HT reuptake inhibitor and releasing agent, was withdrawn from marketing due to safety problems (pulmonary hypertension, valvular heart disease). Further, a recently marketed antiobesity product (sibutramine; Meridia) with a similar mechanism of action Sibutramine, an inhibitor of serotonin and noradrenaline uptake, reduces appetite (inhibition of serotonin and noradrenaline uptake, reducing appetite) is used by only a small

fraction of eligible obese patients because anti-obesity drugs now have a reputation among caregivers and patients as unsafe. Thus approved drugs for the treatment of a disorder that affects many million Americans are only moderately commercially successful because their shortcomings are widely recognized.

5 In summary, medical management of endocrine and metabolic diseases is empirical in nature, is only partially effective, and is associated with multiple undesirable side effects. In view of these clinical realities, the use of genetic tests to identify treatment responders, nonresponders, and/or those likely to develop undesirable side effects will have a major impact on use of existing classes of drugs
10 for treatment of endocrine and metabolic diseases, as well as on the development and use of new drugs to treat these diseases.

G. Cardiovascular and Renal Diseases

The treatment of cardiovascular and renal diseases presents a challenge to
15 physicians and other medical practitioners because the available therapeutics are only partially effective in only a fraction of patients. Further, many currently used medicines produce serious adverse effects. Therapeutic benefits and toxic side effects have to be balanced in each patient. This requires much attention to drug selection, dosage adjustment and monitoring for potential adverse events on the part
20 of care givers – in many cases (e.g. antihypertensive therapeutics) effectively a new pharmacokinetic and pharmacodynamic study must be performed for each patient. These limitations of therapy are especially true of the most debilitating cardiovascular and renal diseases. Although these diseases have distinct clinical presentations, there is extensive overlap in pathogenetic mechanisms and symptoms.

25 Difficulties in treating cardiovascular and renal diseases are attributable to factors such as limited understanding of disease pathophysiology, lack of specificity of pathophysiologic changes (i.e. variation in pathophysiologic mechanisms in patients with similar clinical presentation) and lack of specificity of therapeutic compounds. Further, most medical therapy is directed to the amelioration of
30 symptoms, not the arrest or reversal of underlying pathophysiologic processes.

In summary, medical management of cardiovascular and renal diseases is empirical in nature, is only partially effective, and is associated with multiple undesirable side effects. In view of these clinical realities, the use of genetic tests to identify treatment responders, nonresponders, and/or those likely to develop
35 undesirable side effects will have a major impact on use of existing classes of cardiovascular and renal drugs, as well as on the development and use of new drugs to treat diseases of the cardiovascular and renal systems.

H. Neoplastic Diseases

The unifying feature of neoplastic disease is uncontrolled proliferation and the bulk of modern chemotherapy targets the rapid growth of cancerous tissue. Effective cancer management must destroy or retard the growth of cancerous tissue and prevent the spread of cancerous cells to secondary locations while sparing normal tissues. Cancer therapy has remarkable parallels to the treatment of parasitic infection in that the causative agent is capable of overwhelming growth and rapid mutation to resistant forms. Cancers can differ greatly in their response to chemotherapy: tumors that proliferate rapidly including melanomas, leukemias, and myelomas tend to respond well to classical chemotherapy using cytotoxic agents; tumors that grow slowly, in contrast, such as lung and colon carcinomas tend to respond poorly; the growth of endocrine tumors such as ones of pancreatic, prostate, testicular, ovarian, adrenal, pituitary, or breast origin can be hormonally dependent and treatment with agonists of insulin, estrogen, progesterone, testosterone, etc. function can prove valuable; and solid tumors are more apt to respond to treatment with antiangiogenesis agents than fluid tumors. Surgery (for solid tumors) and radiation treatment exist as therapies that are often used in conjunction with chemotherapeutic agents. A clinician must select a therapy (often a combination of agents and including radiation treatment or surgery) based on tumor type in addition to evaluating the possible toxicities associated with proposed therapeutic regimens, taking the patients current hepatic, renal and myeloproliferative function into account. Since current practice utilizes high doses of cytotoxic agents to minimize the formation of metastases as well as the appearance of secondary, resistant neoplasms, avoiding toxicity becomes a serious issue given the narrow therapeutic index of most drugs in this category.

Medical management of neoplastic disease is empirical in nature, is associated with severe undesirable side effects, and disease progression is common. Based upon these clinical realities and the difficulties medical practitioners face in therapy of neoplastic disease, drug development based upon genotype to identify responders, nonresponders, and or those likely to develop undesirable side effects will be an undeniable beneficial addition to current medical practice.

II. Identification of interpatient variation in response; identification of genes and variances relevant to drug action; development of diagnostic tests; and use of variance status to determine treatment

Development of therapeutics in man follows a course from compound

discovery and analysis in a laboratory (preclinical development) to testing the candidate therapeutic intervention in human subjects (clinical development). The preclinical development of candidate therapeutic interventions for use in the treatment of human diseases, disorders, or conditions begins at the discovery stage
5 whereby a candidate therapy is tested *in vitro* to achieve a desired biochemical alteration of a biochemical or physiological event. If successful, the candidate is generally tested in animals to determine toxicity, adsorption, distribution, metabolism and excretion in a living species. Occasionally, there are available animal models that mimic human diseases, disorders, and conditions in which
10 testing the candidate therapeutic intervention can provide supportive data to warrant proceeding to test the compound in humans. It is widely recognized that preclinical data is imperfect in predicting response to a compound in man. Both safety and efficacy have to ultimately be demonstrated in humans. Therefore, given economic constraints, and considering the complexities of human-clinical trials, any technical
15 advance that increases the likelihood of successfully developing and registering a compound, or getting new indications for a compound, or marketing a compound successfully against competing compounds or treatment regimens, will find immediate use. Indeed, there has been much written about the potential of pharmacogenetics to change the practice of medicine. In this application we provide descriptions of the methods one skilled in the art would use to advance compounds
20 through clinical trials using genetic stratification as a tool to circumvent some of the difficulties typically encountered in clinical development, such as poor efficacy or toxicity. We also provide specific genes, variation in which may account for interpatient variation in treatment response, and further we provide specific
25 exemplary variances in those genes that may account for variation in treatment response.

The study of sequence variation in genes that mediate and modulate the action of drugs may provide advances at virtually all stages of drug development. For example, identification of amino acid variances in a drug target during
30 preclinical development would allow development of non-allele selective agents. During early clinical development, knowledge of variation in a gene related to drug action could be used to design a clinical trial parameters in which the variances are taken account of by, for example, including secondary endpoints that incorporate an analysis of response rates in genetic subgroups. In later stages of clinical
35 development the goal might be to first establish retrospectively whether a particular problem, such as liver toxicity, can be understood in terms of genetic subgroups, and thereby controlled using a genetic test to screen patients. Thus genetic analysis of drug response can aid successful development of therapeutic products at any stage of

clinical development. Even after a compound has achieved regulatory approval its commercialization can be aided by the methods of this invention, for example by allowing identification of genetically defined responder subgroups in new indications (for which approval in the entire disease population could not be achieved) or by providing the basis for a marketing campaign that highlights the superior efficacy and/or safety of a compound coupled with a genetic test to identify preferential responders. Thus the methods of this invention will provide medical, economic and marketing advantages for products, and over the longer term increase therapeutic alternatives for patients.

There are some examples whereby there is no direct evidence that a gene or genes are involved in drug response of a candidate therapeutic intervention. In these cases, however, there is genetic data supporting a role of a variance or variances involved in the etiology, progression, or risk of the neurologic or psychiatric disease. These cases, including but not limited to anxiety, Huntington's disease, demyelinating disease, pain, Parkinson's disease, spasticity, and stroke are described below with details of current therapies and potential genetic involvement of variances in drug responses.

Neurological and Psychiatric Diseases

There are some examples whereby there is no direct evidence that a gene or genes are involved in drug response of a candidate therapeutic intervention for the treatment of neurological or psychiatric disease. In these cases, however, there is genetic data supporting a role of a variance or variances involved in the etiology, progression, or risk of the neurologic or psychiatric disease. These cases, including but not limited to anxiety, Huntington's disease, demyelinating disease, pain, Parkinson's disease, spasticity, and stroke are described below with details of current therapies and potential genetic involvement of variances in drug responses.

A. Anxiety

Description of Anxiety

Anxiety is a common, nonspecific symptom associated to a greater or lesser degree with many psychiatric diseases, including psychoses, neuroses, mood disorders and personality disorders. It is also an inevitable component of everyday life brought on by stressful events such as medical or surgical procedures. Some prominent nonspecific symptoms of anxiety include tachycardia, chest pains, or irregular heartbeat; epigastric distress; headache, dizziness, syncope, or paresthesias. It is usually some combination of these physical manifestations of anxiety that impels patients to seek medical care. It has been estimated that approximately 13% of primary care visits are substantially attributable to anxiety.

There are both acute and chronic anxiety syndromes. The acute forms include panic attacks and event-related anxiety. Chronic, or generalized anxiety is a pervasive feeling of nervousness that does not subside. Because both panic-attack and generalized anxiety lead to desire for being alone or away from public places, many patients adopt agoraphobic tendencies. These patients can become housebound because of fear of having a panic attack in a public setting.

Current Therapies for Anxiety

The principal treatments for anxiety have been benzodiazepines, monoamine oxidase inhibitors, antidepressants, and β -adrenergic antagonists. In all cases, both panic attack and generalized anxiety, concurrent continued behavioral and psychological therapy is required to regain a sense of normal life function.

Limitations of Current Therapies for Anxiety

The difficulty in determining the efficacy of psychotropic drugs for the treatment of anxiety is the subjective contribution of the nonpharmacologic factors that are associated with anxiety. However, the relative safety of benzodiazepines, pharmacologic actions, and high demand make these products drugs of choice in the treatment of anxiety.

The benzodiazepines are associated with side effects due to CNS depression, drowsiness and ataxia. Other side effects are: increase in hostility or irritability, confusion, weight gain, skin rash, nausea, headache, impairment of sexual function, vertigo, and lightheadedness.

Future Drug Development for Anxiety

In Tables 2, 13 and 19, there are listings of candidate genes and specific single nucleotide polymorphisms that may be critical for the identification and stratification of a patient population diagnosed with anxiety based upon genotype. Current pathways that have possible involvement in the therapeutic benefit of anxiety include, but are not limited to, serotonergic, GABAergic, purinergic, adrenergic, glutaminergic, dopaminergic, cholinergic, glycinergic, cholecystokinin, corticotropin releasing factor, histaminergic, opiate, taurine, oxytocin, neuropeptide Y, estrogen, hemostasis, tachykinin, vasopressinergic and second messenger intracellular cascades gene pathways that are listed in Tables 2, 13 and 19. One skilled in the art would be able to identify these pathway specific gene or genes that may be involved in the manifestation of anxiety, are likely candidate targets for novel therapeutic approaches, or are involved in mediating patient population differences in drug response to therapies for anxiety.

Below, Table 26 lists the therapies in development for anxiety categorized by the gene pathway mechanism of action as in Table 7. The listed candidate therapeutic interventions response in patients with anxiety may be affected by polymorphisms in genes as described above.

5 B. Huntington's disease
Description of Huntington's Disease

Huntington's disease (HD) is an inherited disorder characterized by the gradual onset of motor incoordination and cognitive decline in mid-life. Symptoms develop insidiously either as a movement disorder manifested by brief jerk-like
10 movements of the extremities, trunk, face, neck (choreas) or as personality changes. Fine motor incoordination and impairment of rapid eye movements are early features. Bradykinesia and dystonia may predominate if the onset occurs early in life.

As the disorder progresses the involuntary movements become more severe
15 and are characterized by: dysarthria, dysphagia, and impaired balance. Cognitive deficits begin by features of slowed mental processing, difficulty in organizing complex tasks, and memory deficits (family, friends, and immediate situation is unaffected). These patients have tendencies to become irritable, anxious, and clinically depressed. In rare cases there may be paranoia or delusional states. There
20 are approximately 25,000 Americans diagnosed with HD.

Current Therapies of HD

Current therapies do not include alternatives for the treatment of the progression of the neurodegeneration. Medical management of the associated
25 clinical symptoms includes the following categories: depression, psychosis, and choreas. In the cases of depression and psychoses, the therapies of beneficial therapeutic use are described in this invention.

The treatment of choreas generally includes neuroleptic agents that affect dopaminergic pathways by antagonism at the receptor level. Monoamine depleting
30 drugs can also be used to minimize choreas.

Limitations of Current Therapies of HD

Efficacy Limitations

Conventional and atypical neuroleptic agents are not uniformly able to
35 reduce the signs and symptoms of choreas in HD patients. Efficacy varies in the HD population in one or combination of the following ways: 1) patients are only

partially responsive or 2) patients are therapy resistant. Unfortunately, limited efficacy in a HD population in light of the presence of undesirable side effects may lead to compliance issues, aberrant drug abuse behavior, and further safety issues.

Thus, a clinician when presented with a newly diagnosed HD patient, in general, follows standard neurological society or published guidelines for first line therapy. However, when faced with a partially responsive or therapy resistant patient, the clinician can choose from multiple agents, none being completely effective, has limited guidance or rationale to select one agent the other, and follows an empirical medical decision making course of action.

Toxicity Limitations

Unfortunately, conventional neuroleptic drugs are uniformly, and atypical are latently, associated with undesirable dose-dependent side-effects. These include but are not exclusive to sedation, weight gain, cognitive deficits, sexual or reproductive insufficiencies, agranulocytosis, cardiovascular complications, neuroleptic malignant syndrome (parkinsonism with catatonia), jaundice, blood dyscrasias, skin reactions, epithelial keratopathy, seizures, and extrapyramidal effects. The blood dyscrasias include mild leukocytosis, leukopenia, and eosinophilia. The skin reactions include urticaria and dermatitis and are usually associated with phenothiazines. Epithelial keratopathy and opacities in the cornea is associated with chlorpromazine therapy. In extreme cases these effects may impair vision. These ocular deposits tend to spontaneously disappear upon discontinuation of chlorpromazine drug therapy.

The extrapyramidal side effects of conventional neuroleptics include dystonia (facial grimacing, torticollis, oculogyric crisis), akathisia (feeling of distress or discomfort leading to restlessness or constant movement), and parkinsonian syndrome (rigidity and tremor at rest, flat facial expression). With long term usage of conventional neuroleptic drugs, tardive dyskinesias uniformly appear in HD patients.

Tardive dyskinesia is a syndrome of repetitive, painless, involuntary movements. These abnormal involuntary movements are insuppressible, stereotyped, autonomic movements that cease only during sleep, vary in intensity over time, and are dependent on the level of arousal or emotional distress. The syndrome is characterized by quick choreiform (ticlike) movements of the face, eyelids (blinks or spasms), mouth (grimaces), tongue, extremities, or trunk. These movements may have varying degrees of athetosis (twisting movements) and sustained dystonic postures. Increasing the dose of the conventional neuroleptic

agent can reverse extrapyramidal effects observed in patients. However, increasing the dose ultimately leads to more severe dyskinesias. Antiparkinson agents tend to exacerbate the tardive dyskinesia symptoms and thus are not used clinically.

Because dopaminergic agonists tend to worsen the symptoms and dopaminergic antagonists tend to retard the symptoms of tardive dyskinesias, the optimal alternative is to use a neuroleptic agent that has selective dopaminergic antagonist activity. This alternative therapy would manage both psychosis and dyskinesias.

Often a clinician faces the dilemma of a patient with medically managed choreas, but the dose-related tardive dyskinesias, agranulocytosis, or seizures compels the medical care personnel to opt to switch therapies to possibly those agents or drugs with fewer or less severe side effects but with substandard or limited efficacy. Under these conditions, inability to treat the psychotic or chorea symptoms with the backdrop of irreversible dyskinesias leaves the patient with few alternatives.

III. *Impact of Genotyping on Drug Development for HD*

There have been reports of polymorphisms in key genes that affect neuroleptic activity in schizophrenic patients. These polymorphisms may be further applicable for neuroleptic response in HD patients. For example, within the dopamine D4 receptor subtype, there are known tandem repeats in exon 3. In a recent study, schizophrenic patients on maintenance doses of chlorpromazine were stratified into two groups, one having 2 tandem base pair repeats and the other having 4 tandem base pair repeats. Thirty-four percent of group one patients and 62% of group two patients had a favorable response to chlorpromazine therapy during acute stage treatments. The presence of homogeneous four 48 base pair repeats in both alleles in exon 3 of the dopamine D4 receptor subtype thus appears to be associated with beneficial chlorpromazine response.

Recently, a study of the serotonin receptor subtype 6, polymorphism (T267T vs. C267T) in a group of patients refractory to clozapine therapy was reported. In this study, it appeared that the T267T genotype patients were more likely to respond to continued therapy than those patients with C267T genotype patients.

A recent report documented a correlation of the serotonin 5HTC2 receptor subtype biallelic polymorphism and neuroleptic efficacy. A significant number of schizophrenic patients homozygous for the allele C2 responded unsatisfactorily to antipsychotic medication as compared to control.

Three polymorphisms in the serotonergic receptors, i.e. 5HT2A (T102C); 5HT2C (cys23ser); and 5HT2A (his452tyr) have reports of positive or negative correlation with efficacy of antipsychotic therapies. This disparity in the literature

will, in the future, be further examined in schizophrenic patient populations and correlation may be discovered.

V. Description of Mechanism of Action Hypotheses for Future Drug Development

5 The genetic basis of the disease has been identified. A gene, huntingtin, whose protein has a mechanism yet to be defined, has a series of CAG tandem repeats. The number of CAG repeats do correlate somewhat with age of onset and the severity of the disease. Cell death starts in the caudate nucleus by an unknown mechanism. The huntingtin protein is essential to life. The huntingtin protein
10 undergoes cleavage as cells age. The mechanism of cleavage is performed in part by members of the caspase enzymatic family. As the huntingtin protein is cleaved into smaller units, the peptides become toxic, and it has been shown that the smaller fragments tend to migrate into the nuclear compartment. It has been shown that preventing huntingtin cleavage prevents cellular toxicity. Some of the cleaved
15 huntingin fragments form aggregates which may promote or be a by-product of neuronal cell death.

 The profound loss of neurons in the brains of patients with HD has lead to many development programs for the promotion of neural regeneration. These programs broadly include cytokines, growth factors, and agents that promote neural
20 or glial cell growth. Further, consideration of preventing neuronal cell death includes apoptosis inhibition and others. Other programs include prevention of prolonged excitatory neurotransmission. These neurons switch their aerobic metabolism to anaerobic metabolism leading to glycolytic metabolism and excessive production of lactate and metabolic by-products. Excitatory neural inhibition,
25 improvement of energy metabolism, and inhibition of cell death signals may ultimately play a critical role in preventing, retarding, or halting neurodegeneration in HD patients.

 Further, there may be genes within pathways that are either involved in metabolism of neurotransmitters or are involved in metabolism of various drugs or
30 compounds. In Tables 1-6, 12-17, 18-23, there are listings of candidate genes and specific single nucleotide polymorphisms that may be critical for the identification and stratification of a patient population diagnosed with HD based upon genotype. Current pathways that may have involvement in the therapeutic benefit of HD include glutaminergic, serotonergic, dopaminergic, cholinergic, opiates, estrogen,
35 mitochondrial maintenance, growth, differentiation, and apoptosis. secretion gene pathways that are listed in Tables 2, 7, 13, and 19. One skilled in the art would be able to identify these pathway specific gene or genes that may be involved in the manifestation of HD, are likely candidate targets for novel therapeutic approaches,

or are involved in mediating patient population differences in drug response to therapies for HD.

Below in 30 is a list of therapies in development for HD categorized by the gene pathway mechanism of action. The listed candidate therapeutic interventions
5 response in patients with HD may be affected by polymorphisms in genes as described above.

C. The Demyelinating Diseases

Description of Demyelinating disease

10 Primary demyelinating diseases result in loss of the myelin sheath that surrounds axons, with preservation of the axons. The main demyelinating diseases are multiple sclerosis, including its variants (Marburg and Balo variants of MS and neuroromyelitis optica), and the perivenous encephalitides, which include acute disseminated encephalomyelitis and acute necrotizing hemorrhagic leukoencephalitis..

15 Due to the paucity of information concerning etiology of these diseases, identification and classification is largely descriptive. The most common and best studied of these diseases is multiple sclerosis.

Description of Multiple Sclerosis

20 Clinically, MS usually starts as a relapsing illness with episodes of neurological dysfunction lasting several weeks, followed by substantial or complete improvement. This is the relapsing-remitting phase of the disease. Many patients remain in this stage of the disease for years or even decades, while others rapidly progress to the next stage, secondary progressive MS, in which, with repeated
25 relapses, recovery becomes less and less complete. There is also a steadily progressive relapse-independent form of the disease termed primary progressive MS. This form is characterized by a steady worsening of neurological function without any recovery or improvement, and more often affects men.

30 Current Therapies for Multiple Sclerosis

Although the pathogenesis of MS is not understood, there is accumulating evidence that immunoregulatory mechanisms are involved. Current therapy of MS is therefore directed to modulating immune function and thereby halting or retarding myelin degeneration, or facilitating remyelination. Remyelination has been shown
35 to occur spontaneously in response to therapeutic interventions in animals (both normals and MS models). However, in MS animal models remyelination appears to be aborted soon after it begins.

For relapsing-remitting MS the following agents are currently in use : 1) interferon beta-1 β (Betaseron) reduces annual relapse rate and reduces development and progression of new lesions in relapsing-remitting MS as monitored by magnetic resonance imaging (MRI), and has been shown to reduce annual relapse rate, reduce disability progression, and delay increase of lesion volume by MRI in secondary progressive MS; 2) Interferon beta-1 α (IFN-beta-1 α ; Avonex) treatment results in reduced disability progression, annual relapse rate, and new brain lesions, as visualized by MRI; 3) Glatiramer acetate (Copaxone; Copolymer-1; Cop-1) reduces annual relapse rate; 4) Intravenous immunoglobulin, reduces annual relapse rate, and delays disability progression; 5) High-dose methylprednisolone therapy is effective in shortening MS attacks, and may be useful in the long term treatment of secondary-progressive MS; 6) Other agents that have been used with success are mitoxantrone, azathioprine and methotrexate. The latter drug, in particular, has been shown effective in reducing disease activity, both by decreasing the number of exacerbations and by slowing clinical progression. The first four agents are of comparable efficacy in the treatment of relapsing-remitting MS. Not enough trials have been performed to reliably assess the utility of treating nonresponders to one of these treatments with a different treatment, or to assess potential markers of response.

III. Limitations of Current Therapies for Multiple Sclerosis

The available treatments have both efficacy and toxicity limitations. Further, the cost for one year of interferon treatment is approximately \$11,000 and parenteral administration is inconvenient.

Partial Response to Therapy

Current therapies reduce, but do not arrest, disease progression, and only a fraction of patients benefit from treatment; approximately 30% of patients on interferons experience reductions in relapse rates. For primary progressive MS, there are currently no effective therapies available; interferon beta-1b has in fact been shown to worsen spasticity in primary progressive MS.

Undesired Side-Effects or Toxicities as a Therapeutic Limitation

All interferons are associated with varying degrees with flu-like symptoms, muscle-ache, fever, chills, and asthenia. There are also side effects that are difficult to distinguish from the course of the demyelinating illness, for example interferons may lower the seizure threshold and exacerbate depressive illnesses, two clinical problems also observed in patients without interferon therapy.

Impact of Pharmacogenomics on Drug Development for Multiple Sclerosis

Aspects of therapy for demyelinating disease that can be addressed by pharmacogenetic methods include: 1) Which patients are most likely to respond to medication? 2) Which drugs are most likely to benefit which patients? 3) What is the optimal dose and duration of treatment? 4) What is the relationship between disease type, stage and manifestations and drug response? 5) Can adverse treatment responses be predicted? As an alternative to directly correlating genetic variants with clinical responses to therapy, one could also use quantitative biochemical, immunological or anatomical measures of disease activity to assess the impact of genetic variation in candidate genes on response to medication. While it is unlikely that all therapeutic responses are under strong genetic control, it is expected that if stratification based upon genotype were performed in clinical trials a correlation between drug response and genotype will be detected for at least some treatment responses. Described below and in Tables 2 and 7 are gene pathways that affect current drug therapy as well as drugs currently in development for MS. Described in the Detailed Description are methods for the identification of candidate genes and gene pathways, patient stratification, clinical trial design and statistical analysis and genotyping for testing the impact of genetic variation on treatment response in multiple sclerosis and other demyelinating diseases.

A sample of therapies approved or in development for preventing or treating the progression of symptoms of MS currently known in the art is shown in Table 32. In this table, the candidate therapeutics were sorted and listed by mechanism of action. Further, the product name, the pharmacologic mechanism of action, chemical name (if specified), and the indication is listed as well.

Mechanism of Action Hypotheses for Novel Therapies for Multiple Sclerosis: Utility of Genotyping

Several possible mechanisms by which intravenous immune globulin (IVIG) modulates the course of the disease are related to limiting the inflammatory process and repairing the damage by enhancing remyelination. The efficacy of dexamethasone (DX) and methylprednisolone (MP) at high (HD) and low (LD) dose in acute multiple sclerosis (MS) relapses was evaluated by a double-blind trial in 31 patients followed for 1 year. DX and HDMP were similarly efficacious in promoting recovery, while LDMP was ineffective in the short-term outcome and was followed by an early clinical reactivation. The different outcomes seem to be related to different immunomodulating effects, mainly on cerebrospinal fluid (CSF) IgG

synthesis and on peripheral blood and CSF CD4+ lymphocyte subsets. The efficacy of interferon should be investigated in relation to other treatment options, such as immunoglobulin, copolymer I, azathioprine and methotrexate. Other promising therapeutic options (mitoxantrone, intravenous immunoglobulins, drug associations) are under evaluation.

Pathogenesis of MS

There are three current theories for the cause of MS that have been studied to effectively understand the mechanism of disease as well as establish rationale for the development of effective candidate therapeutic interventions. The three current theories are 1) viral infection, 2) genetic predisposition, 3) inflammation and autoimmunity, and 4) ion channel modulators.

Viral Infection

Indirect evidence that there is a single unique virus causing MS is the unusual geographic distribution of the disease. There is a documented north-south gradient of disease prevalence, migration studies, and reports of clustering of cases have indicated an environmental influence on disease susceptibility. Despite years of intense research including viral isolation studies from tissue samples of MS patients and controls, has not resulted in identification of an MS specific virus or viral sequence.

One virus implicated in the pathogenesis of MS is the human herpes virus type 6 (HHV-6). HHV-6 is a neurotropic virus that can establish a latent infection in man. HHV-6 protein and DNA have been isolated and identified from neuroglial cells in active MS spinal lesions. Further, HHV-6 IgM titers in MS patients and HHV-6 DNA identified in serum samples indicate a recent infection. However, to date there is no evidence that HHV-6 is the causal infectious agent of MS. Instead, a hypothesis of molecular mimicry has been proposed as a likely possibility to explain the indirect immune-mediated injury to otherwise normal tissue in the course of clearing an infectious agent. Besides HHV-6, there are other neuro-specific infectious agents that may damage the CNS through this mechanism. The molecular similarity (mimicry) between virus and myelin antigens may be permissive for immunological cross-reactivity between HHV-6 and myelin antigens. In this model, the T-cells become activated, cross the blood brain barrier and misidentify normal myelin antigens as 'virus' resulting in T-cell mediated cellular and tissue injury.

Genetic Susceptibility to MS

Although MS is a sporadic disease, studies have pointed to an organized familial clustering, which suggests a genetic predisposition to MS. Equally likely, these studies also suggest that there is a genetic predisposition to an environmental stress or causal event.

The most convincing evidence of a genetic predisposition to MS is derived from studies of a population-based study of twins. The risk of MS increases with the degree of shared information within a family. There is further a marked increase in concordance for MS in the comparison of monozygotic and dizygotic twins.

Inflammation and Autoimmunity in MS

While it is clear there is an inflammatory component to the lesions of MS, is it currently unclear whether the immune system plays a role in initiation of the characteristic damage of white matter.

In experimental studies of animal models of MS, there appears to be T-cell, CD4+ and CD8+, autoreactivity to several putative CNS antigens including myelin basic protein, proteolipid protein, myelin oligodendroglial glycoprotein, 2',3'-cyclic nucleotide phosphodiesterases, myelin-associated glycoproteins, and viral antigens. Further, there appears to be down-regulation of cytokine production including TNF- α and IL-3.

These observations have led to the following proposed mechanism of immune-mediated injury in an MS lesion. Genetic and environmental factors (e.g. viral infection, molecular mimicry, bacterial lipopolysaccharides, superantigens, local metabolic stress, oncogene expression, or reactive metabolites) may potentiate the movement of T-cells through the blood-brain barrier to the CNS. These same genetic and environmental factors may act within the CNS to upregulate the expression of intracellular adhesion molecules on endothelial cells and the circulating T-cells which in turn enhances the rolling, binding, diapedesis, and ultimate migration of the T-cells into the CNS. The same genetic and environmental factors may activate the secretion of $\alpha\beta$ -crystallin on the oligodendrocytes rendering these cells more susceptible to T cell recognition. The T-cells once in the CNS then secrete cytokines (TNF- β and INF- γ) activate the antigen presenting cells (astrocytes, microglia, and macrophages) enhancing (macrophage, microglia) or inhibiting (astrocytes) further immune signaling. The activated T cell then encounters the putative MS antigen or antigens in light of the MHC class II molecules on the antigen presenting cells, resulting in T-cell activation. The activated T-cells can then differentiated into Th1 or Th2 type CD4+ cells which then results in proinflammatory or anti-inflammatory cytokine signaling, respectively. It

has been shown in MS patients that antibody, complement, and antibody-mediated cellular toxicity mechanisms may cause the myelin lesions.

Ion Channel Modulations in MS

Reduction of the depolarization in postsynaptic membranes by modulation of the ion channels in nerve and muscle tissue has been postulated as a mechanism to ablate aberrant neurotransmission in demyelinating neurological disease. Proposed gene targets to produce the membrane depolarization are the nicotinic acetylcholine receptor, voltage gated Na⁺ channels, and other ion channels.

Future Therapeutic Strategies for MS

The future strategies for the beneficial therapy of MS are borne out of the existing mechanisms of the etiology of this demyelinating disease as previously described. They are antivirals, cytokine and anticytokine strategies, immune deviation strategies to enhance Th2 cell/cytokine performance, matrix metalloproteinase inhibitors, trimolecular complex strategies, cathepsin B inhibitors, and oxygen radical scavengers.

Specifically, antivirals include valacyclovir and acyclovir. Cytokine and anticytokine strategies include TNF inhibitors, antiinflammatory cytokines, and inhibitors of proinflammatory cytokines. Immune-deviation strategies to enhance Th2 cell/cytokine predominance includes pentoxifylline, transforming growth factor- β (TGF- β), and IL-10, IL-4 alone in combination with corticosteroids. Matrix metalloproteinase inhibitors include D-penicillamine, and hydroxyaminate. Trimolecular complex strategies include anti-MHC monoclonal antibodies, MHC class II hypervariable peptide vaccines, anti-T cell monoclonal antibodies, altered peptide ligands, T cell vaccination strategies (myelin basic protein reactive T-cell, T-cell receptor peptide vaccination), co-stimulation strategies (antiB7-1, CTLA-4Ig fusion proteins, CD40/CD40 ligand interactions), and adhesion molecule signaling strategies (monoclonal antibodies, or small molecules directed to these adhesion molecules).

Neural regeneration development programs will include growth factors including NGF, BDGF, CNTF, NT-3, and other cytokines, as well as other factors that are involved in the support of nerve cell viability, growth, and sustaining neural transmission.

Technological advances that reduce difficulties in determining progression of the demyelination by neuroimaging techniques will aid development of new therapies. Estimation of expected clinical and surrogate measures and patterns to

identify, screen, and develop statistically derived stopping rules for efficacy and futility.

Further, there may be genes within pathways that are either involved in metabolism of neurotransmitters or are involved in metabolism of various drugs or compounds. In Tables 2, 13, 19 there are listings of candidate genes and specific single nucleotide polymorphisms that may be critical for the identification and stratification of a patient population diagnosed with MS based upon genotype. Current pathways that may have involvement in the therapeutic benefit of epilepsy include glutaminergic, GABAergic, opiates, corticotropin releasing hormone, potassium channel, prostaglandin, platelet activating factor, cytokines, clot formation, second messenger cascade, growth, differentiation, and apoptosis, cytoskeleton, adhesion, and myelination gene pathways that are listed in Tables 2, 7, 13, and 19. One skilled in the art would be able to identify these pathway specific gene or genes that may be involved in the manifestation of MS, are likely candidate targets for novel therapeutic approaches, or are involved in mediating patient population differences in drug response to therapies for MS.

D. Pain

Description of Pain

Chronic pain can be caused by chronic pathologic processes in somatic structures or viscera, or by prolonged dysfunction of parts the peripheral or central nervous system.. In all there are approximately 70 million Americans that experience chronic pain. Chronic pain may be the result of recurrent headache, arthritis, back or spinal injuries, musculoskeletal disorders, cardiac or visceral pathologies. Chronic pain is also part of the clinical manifestation of cancer; many of these cases are medically intractable pain. Chronic pain syndromes include polyarteritis nodosa; systemic lupus erythmatosus; entrapment neuropathy; lumbar plexitis; Bell's palsy; carpal tunnel syndrome. Chronic pain can also result from peripheral neuropathies: diabetic neuropathy (neuropathic complications of diabetes mellitus include distal symmetric, sensory, autonomic, asymmetric proximal, cranial and other mononeuropathies); cervical radiculopathy; Guillain-Barre syndrome; brachial plexitis; familial amyloid neuropathy; HIV neuropathy; post spinal cord injury; and post herpetic neuralgia.

Current therapies for Pain

Therapeutic management of chronic pain includes a three step ladder approach: non-opioid analgesics are stepwise prescribed in combination with moderate to potent opiates. The guidelines call for a determination by the patient

and the physician of pain relief. Broadly speaking, the guidelines are as follows: mild pain is treated with non-opioid analgesics, moderate or persisting pain is treated with a weak opioid plus non-opioid analgesics, and severe pain that persists or increases is treated with a potent opioid plus non-opioid analgesics.

5 Pain management regimens include not only the use of opioids and non-opioid analgesics, but also benzodiazepines, local anesthetics, anticonvulsants, anticholinergics, serotonin norepinephrine reuptake inhibitors, neuroleptics, and barbiturates. These drugs in combination can relieve associated symptoms of chronic pain syndromes such as anxiety, acute on top of chronic pain, seizures, dry
10 mouth, delirium, and inability to sleep, respectively.

Treatment options for chronic pain fall into the following categories: 1) general health promotion and relief from exacerbating factors; 2) nonnarcotic pharmacologic; 3) physical; 4) surgical; and 5) narcotic.

The nonnarcotic empirical therapies include tricyclic antidepressants
15 (amitriptyline, nortriptyline, doxepin, imipramine), anticonvulsants (carbamazepine, phenytoin); GABAergic agonists (BACLOFEN[®]) and antipsychotics (fluphenazine). Narcotic therapies include opioid agonists (methadone and fentanyl). Devices and surgical therapies can be used in combination with drug therapy. In general these therapies have been shown to reduce pain and each are described in detail below.

20 Antidepressants: The tertiary amines are the most commonly used anti-depressants to manage pain associated with post-SCI. Although the exact mechanism is unknown, the interference with the re-uptake of neurotransmitters (dopamine, norepinephrine, and serotonin) may reduce pain transmission in the afferent
25 pathways. Further, the increased quantities of these neurotransmitters in the areas of the hyperexcitable neurons, descending pain inhibitory pathways that terminate in the substantia gelatinosa of the dorsal horn, may act to reduce pain transmission. Interestingly, the dose of the tricyclics for the management of pain is approximately half that required for the management of depression. These compounds can be
30 determined to be effective for pain management in approximately two weeks.

Anticonvulsants: Reports exist describing chronic neuropathic pain syndromes as a central neurophysiologic epileptiform activity of the uncontrolled hyperactive
35 neurons leading to a convulsive syndrome in the spinal cord. Thus, anticonvulsant therapies are considered to stabilize the threshold against hyperexcitability of neurons and inhibiting the spread of epileptiform activity in neurons involved in nociception. Further, activation of inhibitory neurons may lead to a pain reduction.

Although the data is not conclusive, it appears that anticonvulsants are more effective when given in combination with antidepressants.

5 Neuroleptics: The neuroleptics are thought to exert a potentiation of the antidepressants and may impart a dopaminergic antagonism. Neuroleptics are usually given in combination with an antidepressant.

10 GABAergic agonists: Baclofen, a GABAergic agonist when delivered intrathecally was effective in reducing chronic pain in those patients in which the pain was of musculoskeletal origin (83% of these patients), but was ineffective in those patients with neurogenic pain (78% experienced no change).

15 Physical treatments: Physical treatments include transcutaneous electrical nerve stimulation (TENS) and spinal cord stimulation devices. Using TENS, some success has been reported to reduce peripheral pain. Upon placing the electrodes, peripheral sensory nerve stimulation is thought to activate pain inhibitory interneurons in the substantia gelatinosa or dorsal root entry zone of the spinal cord. Spinal cord stimulation devices are programmable multichannel systems with electrodes that may be placed percutaneously; these systems do not require
20 laminectomy. These stimulators have been shown to reduce chronic pain (perceived pain levels requiring intensive therapies: discomforting, distressing, horrible, and excruciating) by 50% long term. The global ratings for quality of life in these patients demonstrated similar long term improvements. The exact mechanism of how spinal cord stimulation results in a reduction of pain is unknown, but it is
25 thought to occur through an antisympathetic effect. Further, it seems to be effective in cases in which the patient has neuropathic or an ischemic component to the pain. In patients with peripheral neuropathies (postherpetic neuralgia, intercostal neuralgia, causalgic pain, diabetic neuropathy, idiopathic neuropathy) spinal cord stimulation is able to reduce chronic pain in approximately 50% of the patients.

30 Surgical treatment: If conservative pharmacologic approaches have failed to relieve pain, neurosurgery can be considered. Neurosurgical treatments consist of nerve blocks, neuroablative and neuroaugmentative procedures.

35 Nerve blocks: Peripheral, epidural, and sympathetic nerve blocks have been attempted. However, the analgesic effect is usually short-lived and ineffective against central mechanisms of pain.

Neuroablative procedures: There are surgical procedures that are rarely performed because they have been shown to be ineffective, i.e. sympathectomies, neurolyses, dorsal rhizotomies, cordectomies, anterolateral cordectomies, mesencephalotomies, and cingulotomies. These procedures have been superseded by dorsal root entry zone (DREZ) surgery. The surgical procedure involves a laminectomy of the appropriate vertebrae, examination of the DREZ and radiofrequency lesions of the DREZ. The mechanism of this ablative surgery is thought to be due to the destruction of the secondary pain sensory neurons in the substantia gelatinosa in the dorsal horn. Success of this procedure on the reduction of pain has been reported at 60-90%.

Neuroaugmentative procedures- deep brain stimulation: Electrodes are implanted in the periventricular gray matter, specific sensory thalamic nuclei, or the internal capsule.

Limitations of Current Therapies of Pain Due to Low Efficacy

The severity of pain can be debilitating and significantly interfere with the productivity and quality of life. Existing therapies for chronic pain are often inadequate and characterized by the tendency to become ineffective with time.

Potent opiates are part of an analgesic regimen, however, dose-limiting side effects and antinociceptive capacity, tolerance and potential for dependence limit their widespread use. Surgical intervention is sometimes attempted, but often such procedures are ineffective and at best provide only temporary relief.

There are many syndromes by which the above combination drug therapy is insufficient to relief symptoms of chronic pain. There are common reasons for unrelieved pain associated with the patient or family, i.e. belief that pain in cancer is inevitable and untreatable, failure to contact a physician, patient denial, failure to take medications, noncompliance due to fear of addiction, noncompliance due to a belief that tolerance will rapidly develop and adequate pain relief then will not be available in the advanced stages, and lastly noncompliance due to the adverse side effects. Common reasons for unrelieved pain associated with the physician or nurse are: denial of the patient's pain, unawareness of pain intensity, failure to perceive patient denial, failure to treat pain aggressively, fear of patient addiction, failure to prescribe appropriate doses for analgesia, failure to monitor the patient's progress, failure to understand alternative drug combinations, and finally failure to give psychological support to the patient and family. Despite these common reasons for unrelieved chronic pain, even under positive conditions chronic pain can be intractable in a variety of diseases.

The coexistence of pain and depression in these patients is a dependent relationship, i.e. when the pain is unmanaged the depression becomes more severe, the reverse (increased depression leads to increased pain) relationship is less likely to occur. The characteristic intensity of the pain and psychological impact prompts extreme potential solutions. Some of these pain syndromes are more resistant to analgesic therapy, for example approximately half of the individuals with spinal cord injuries endure chronic pain and 30% experience severe, debilitating chronic pain. Approximately 75% of advanced stage cancer patients experience moderate to severe pain and approximately half of these individuals are refractory to standard therapy for management of pain.

Other efficacy limitations include: slow onset of symptoms (2-3 weeks) before efficacy detection for tricyclic antidepressants.

Limitations of Current Therapies of Pain Due to Toxicity or Undesired side effects

In the stepwise approach to therapy, physicians are able to monitor and adjust the doses to limit undesired side effects of opioids: sedation, cognitive impairment, myoclonus, addiction, and respiratory depression. Further, opiate tolerance is a well documented effect seen in routine narcotic users and abusers. These side effects may provoke a use of opioid rotation in a pain management schedule.

Although the use of opioids in acute and chronic cancer associated pain is well accepted, their use in chronic noncancer pain has been widely considered to be inappropriate due to concerns over efficacy, toxicity and addiction.

Other unwanted or undesirable side effects include tardive dyskinesias limit the use of neuroleptics in the management of chronic pain; oral baclofen is associated with drowsiness and confusion. Further, baclofen may cause hepatotoxicity. Complications of radiofrequency lesions of DREZ procedure includes cerebrospinal fluid leaking, loss of sensory/motor functions, exacerbation of bowel, bladder, or sexual dysfunction, and epidural/subcutaneous hematomas. Patients must consider the risks of this procedure, particularly the potential loss of two levels of sensation. Associated with deep brain stimulation are complications due to the release of large amounts of natural opioids leading to deafferentation and nociceptive pain.

Impact of Genotyping on Drug Development for Pain

As described above, there is evidence to suggest that there are efficacy and safety differences to drug therapy in the pain patient population. Although not all of these responses may be attributable to genotypic differences, it is expected that if

stratification based upon genotype were performed, a reasonable correlation between drug response and genotype may become obvious. As described below, there are gene pathways that are involved with current drug therapy and those that may be potentially involved in the future. As described in the Detailed Description, methods for the identification of candidate genes and gene pathways, stratification, clinical trial design, and implementation of genotyping for appropriate medical management of a given disease is easily translated for pain syndromes. As described below in section V. below there are likely gene pathways as are those that are outlined in the gene pathway Table 2 and CNS matrix table 7.

For example, optimization of GABAergic, opiate, or ion channel modulation mediated therapy of pain further demonstrates the utility of selection of a potential epilepsy patient that has a predisposing genotype in which selective analgesics or agents are more effective and or are more safe. In considering an optimization protocol, one could potentially predetermine variance or variances within the GABAergic receptor, ion channel or ion channel mediated mechanisms of neurotransmission, or GABAergic receptor mediated intracellular mechanism of action that is preeminently responsible for drug response. By embarking on the previously described gene pathway approach, it is technically feasible to determine the relevant genes within such a targeted drug development program for pain.

A sample of therapies approved or in development for preventing or treating the progression of symptoms of pain currently known in the art is shown in Table 33. In this table, the candidate therapeutics were sorted and listed by mechanism of action. Further, the product name, the pharmacologic mechanism of action, chemical name (if specified), and the indication is listed as well.

Description of Mechanism of Action Hypotheses for Future Drug Development for Pain

The persistence of pain most likely involves a cascade of pathological neurochemical events that lead to abnormal sensory hyperexcitability and excitotoxicity. The persistence of hyperexcitability involves a sequence of neuroplastic events in the spinal cord. In particular, the hyperexcitability cascade involves NMDA receptor mediated intracellular calcium-dependent increase of nitric oxide (NO) and cGMP production. These signals facilitate long-term alterations in neuronal excitability and central sensitization. The altered spinal neurochemical environment results in an impairment of neural inhibitory function. In particular, inhibitory gamma-aminobutyric acid (GABA)-ergic interneurons are susceptible to excessive excitatory amino acid release. Recent studies suggest that abnormal pain sensations may be alleviated by application of GABA receptor

agonists. The analgesic capacity of GABA receptor agonists has been demonstrated in numerous animal models of acute and chronic pain.

Further, there may be genes within pathways that are either involved in metabolism of neurotransmitters or are involved in metabolism of various drugs or compounds. In Tables 2, 13, and 19, there are listings of candidate genes and specific single nucleotide polymorphisms that may be critical for the identification and stratification of a patient population diagnosed with pain based upon genotype. Current pathways that may have involvement in the therapeutic benefit of epilepsy include glutaminergic, serotonergic, dopaminergic, adrenergic, cholinergic, histaminergic, purinergic, GABAergic, glycinergic, melatonin, nitric oxide, peptide protein hormone processing, opiates, cholecystokinin, tachykinin, bradykinin, corticotropin releasing hormone, somatostatin, galanin, calcium or sodium channels, prostaglandin, cytokines, growth, differentiation, apoptosis, lipid transport/metabolism pathways that are listed in Tables 2, 7, 13, and 19. One skilled in the art would be able to identify these pathway specific gene or genes that may be involved in the manifestation of pain, are likely candidate targets for novel therapeutic approaches, or are involved in mediating patient population differences in drug response to therapies for pain.

E. Parkinson's Disease

20 Description of Parkinson's Disease

Parkinson's disease (PD) is one of the major neurodegenerative disorders of middle and old age. PD is a clinical syndrome that is dominated by four clinical symptoms: tremor at rest, bradykinesia, rigidity, and postural instability. There are secondary clinical signs and symptoms also associated with PD and are a result of the following manifestations: mood and intellectual disorder, oculomotor control, and autonomic and sensory dysfunction. PD can be generally categorized by the clinically predominant parkinsonian feature: 1) those patients having tremor, or 2) those patients having postural instability and or gait difficulty as the predominant clinical parkinsonian manifestation. In those patients with tremor predominant disease, the onset is earlier in life and exhibits a slower progression than those patients with gait difficulties or postural instability. In the latter case, the age of onset is later in life and is more frequently associated with bradykinesias, dementia, and the movement disorder progresses more rapidly. The stages of PD have been described and are referred to as Hoehn and Yahr stages I through V; stage I- signs and symptoms are unilateral, stage II- signs and symptoms are bilateral, stage III- signs and symptoms are bilateral and balance is impaired, stage IV- functionally disabled, and stage V- patient is confined to wheelchair or bed.

Resting tremor and bradykinesias are the hallmarks of PD. Bradykinesias are primarily responsible for the altered clinical presentation for most PD patients: retardation of activities of daily living and generalized slowing down of movements, lack of facial expression (hypomimia or masked facies), staring expression due to limited ability to blink, impaired swallowing which causes drooling, hypokinetic and hypophonic dysarthria, monotonous speech, micrographia, impaired simultaneous and repetitive movements, difficulty in standing from a chair and turning in bed, shuffling gait with short steps, decreased arm swing and other autonomic movements, start hesitation and sudden freezing of motion. Freezing of motion manifests as a sudden and often unpredictable inability to move and represents the single most disabling parkinsonian symptoms.

There are several disorders other than PD that manifests with parkinsonian symptoms. For example, acquired or symptomatic parkinsonism is the result of infectious (postencephalitic and slow virus) disease, side effects from drugs (neuroleptics (antipsychotic and antiemetic drugs), reserpine, tertabenazine, amethyl dopa, lithium, flunarizine, cinnarizine), toxins (MPTP, carbon dioxide, manganese, mercury, cesium, methanol and ethanol), cerebrovascular insult (multi-infarct, hypotensive shock), trauma (pugilistic encephalopathy), and others (parathyroid abnormalities, hypothyroidism, hepatocerebral degeneration, cerebral tumors, normal pressure hydrocephalus, syringomesencephalia). Parkinsonism can also be the result of hereditary degenerative disease, for example autosomal Lewy body disease, Huntington's disease, Wilson's disease, Hallervorden-Spatz disease, olivopontocerebellar and spinocerebellar degenerations, familial basal ganglia calcification, familial parkinsonism with peripheral neuropathy, and neuroacanthocytosis. Lastly, parkinsonism can be the result of multiple-system degenerations and include for example progressive supranuclear palsy, Shy-Drager syndrome, striatonigral degeneration, Parkinsonism-dementia-amyotrophic lateral sclerosis complex, corticobasal ganglionic degeneration, Alzheimer's disease, and hemiatrophy-parkinsonism. These non-PD parkinsonism symptoms can be clinically identified as distinct from PD due to the presence of atypical signs or symptoms of the particular dysfunction or syndrome, absence or paucity of tremor, and poor response to levodopa.

Current Therapies for PD

Pathophysiologically, idiopathic PD cases are almost uniformly identified by the absence of dopaminergic terminals and depigmentation within the substantia nigra and the presence of Lewy bodies (eosinophilic cytoplasmic inclusions in neurons consisting of aggregates of normal filaments). These abnormalities are

predominantly found in the ventrolateral region of the substantia nigra which is the region that projects to the putamen. It has been estimated that at least 80% of dopaminergic neuronal loss within the substantia nigra and an equal degree of dopamine depletion within the striatum is required before signs and symptoms of PD is clinically observed.

There are currently four categories of drug therapies for the treatment of PD: dopaminergic replacement drugs, dopaminergic agonists, anticholinergic drugs, and monoamine oxidase inhibitors. Other therapies include surgical treatment and implantable devices for control of debilitating essential tremor.

Dopaminergic Replacement Drugs- therapy of PD is aimed at replacing the lost dopamine that has resulted in the loss of dopaminergic neurons in the substantia nigra and other brain regions. L-dopa is a prodrug that can be converted to dopamine within the existing neurons. Generally, L-dopa is beneficial in early PD, because it is effectively metabolized in presynaptic terminals and secreted in an active form. Due to the rapid decarboxylation of L-dopa in the periphery, administration of large doses is required to achieve therapeutic benefit. However, L-dopa is usually administered with carbidopa, an inhibitor of peripheral decarboxylation and thus greater concentrations of L-dopa enters the CNS. The combination of L-dopa and carbidopa reduces by 75% the amount of L-dopa required.

Dopaminergic Agonists- dopaminergic agonists can be administered in the early stages of the disease, examples include pramipexole and pergolide.

Anticholinergic Drugs- anticholinergic agents are prescribed for the management of tremor or inordinate movements associated with PD, examples include trihexyphenidyl, and benztropine. The majority of the anticholinergic therapies for the adjunct treatment of PD are long-acting medications thus relief of symptoms may continue through the night when patients have difficulty turning in their bed, and to rise in the morning.

Monoamine Oxidase Inhibitors- inhibition of the metabolism of dopamine by monoamine oxidase can be achieved to increase the synaptic levels of dopamine. An example is selegiline.

Others- catechol-o-methyl transferase inhibitors may be prescribed for the adjunctive treatment of PD, example is Tasmar. An antiviral, symmetrel, has been used for the relief of tremors, rigidity, and bradykinesia. Some β -adrenergic antagonists have been shown to reduce tremors, example is propranolol.

Prior to the advent of levodopa therapy, the most effective means of treating disabling tremors associated with PD were thalamotomy and pallidotomy. These ablative surgical procedures are associated with improved tremor and in certain cases, bradykinesias. Recent advances in neurosurgery, e.g. devices to specifically record from the globus pallidus for enhanced localization, have been employed and there is renewed clinical interest in considering these therapies for the treatment of PD. This therapy has the advantage of single procedure therapeutic intervention of disabling tremors.

Another therapeutic alternative for the treatment of essential tremor, a device for deep brain stimulation, is approved for unilateral implantation in the ventral intermediate nucleus of the thalamus. A programmable, implantable pulse generator is implanted just below the clavicle. The implanted device has been shown to be effective in 20% of the patients, bilateral implantation and stimulation is under investigation.

Limitations of Current Therapies for PD

Although there are therapeutic alternatives for the early intervention of PD, there are few alternatives for the later stages and for the side effects that develop after long term therapy. These limitations are discussed below.

Limitations of Current Therapies due to Low Efficacy

All anti-Parkinson drugs have two qualities that limit the efficiency of treatment regimens. First, the drugs are relatively short acting. A single administration does not relieve symptoms for the duration of waking hours, and multiple administrations are required. The second is that these drugs are all centrally acting drugs and starting dosage is low and slowly increased. Abrupt withdrawal or reductions of any of these medications can lead to deleterious side effects.

L-dopa therapy of PD has therapeutic benefit in the early stages of the disease. However, as the movement disorder progresses, the dopaminergic terminals are lost and the prodrug is no longer converted to the active form. The therapeutic benefit is then limited to the level and extent of the intact postsynaptic neurons.

Long-term therapy with levodopa is associated with dose dependent side effects including inefficacy, "on-off" phenomena, and dyskinesias. Response fluctuations occur in approximately 80% of the patients. These fluctuations consist of wearing-off phenomena, a gradual loss of effectiveness of levodopa related to the timing of administration of the drug, and the on-off phenomena, which is an abrupt

loss of the effectiveness of levodopa that is not related to the timing of administration.

Dyskinesias, consisting of chorea and dystonia, occur in approximately 40% of patients treated with levodopa. These dyskinesias are most frequently observed when plasma levels of L-dopa are high. For patients with preexisting history of psychiatric illness, anticholinergic therapies are less likely to be administered and further if prescribed are less likely to be effective. Thalamotomy and pallidotomy are two surgical procedures that can only be performed once per side. Thus, refractory cases or cases whereby surgery was not sufficient to alter the essential tremor, additional surgery is unavailable. Deep brain stimulation is only 20% effective, requires extensive follow-up, and is associated with a surgical morbidity of 5%. Animal model studies of growth factors, GDNF, affected sprouting of peripheral neurons and those in the spinal cord. Unregulated neural sprouting can be deleterious to neurological function.

Limitation of Current Therapies due to Toxicity or Undesired Side Effects

Limitations due to toxicity or undesired side effects for the above discussed treatments of PD are as described below for each of the treatment strategies.

Dopaminergic replacement drugs- as described above, L-dopa is a prodrug that can be of therapeutic benefit to patients with PD. However there are side effects and toxicities associated with L-dopa therapy, they are choreiform and dystonic dyskinesias and other involuntary movements, adverse mental changes such as paranoid ideation, psychotic episodes, depression, and cognitive impairments (dementia). Dyskinesias associated with levodopa, can be debilitating and as uncomfortable as the rigidity and akinesia of PD.

Reductions or withdrawals of L-dopa therapy have been associated with neuroleptic malignant syndrome (NMS). NMS is an uncommon but life-threatening syndrome characterized by fever or hyperthermia, muscle rigidity, involuntary movements, altered consciousness, autonomic dysfunction, tachycardia, tachypnea, sweating, and hyper- or hypotension.

Dopaminergic agonists- as described above, dopaminergic agonists are useful for the activation of post synaptic dopaminergic receptors. The side effects and toxicities associated with dopaminergic agonists are: abnormal involuntary movements, hallucinations, "on-off" phenomena, dizziness, fainting, visual disturbances, ataxia, insomnia, depression, hypotension, constipation, vertigo, and shortness of breath. It

has been observed clinical laboratory transient elevations of blood sera urea and nitrogen, SGOT, SGPT, GGPT, CPK, alkaline phosphatase, and uric acid.

Anticholinergic drugs- the predominant affect afforded by the anticholinergic drugs is to treat the extrapyramidal effects that develop with long-term dopaminergic therapies. This therapy is thus via the anticholinergic and antihistaminergic effects. However, there are adverse reactions that are associated with anticholinergic therapies, they are tachycardia, paralytic ileus, constipation, dry mouth, toxic psychosis (confusion, disorientation, memory impairment, visual hallucinations, possible exacerbation of preexisting psychiatric symptoms or syndromes, blurred vision, dysuria, and urinary retention.

Monoamine oxidase inhibitors- selective inhibition of monoamine oxidase type B (MAO-B) enzyme activity is a useful adjunctive therapy to increase concentrations of dopamine in regions of the brain. Since MAO-B is predominantly found in the brain, fewer systemic side effects occur. Despite this selectivity, there are side effects that are undesirable, they are exacerbation of L-dopa or other dopamine agonist mediated side effects. For example, dyskinesias are enhanced as well as the others listed above.

MAO-B inhibition can be deleterious if administered with a tricyclic antidepressant. Further, a combination of MAO-B inhibitor and meprobamate (an opioid narcotic) has lead to stupor, muscle rigidity, severe agitation, and hyperthermia. Thus, concomitant administration of these two types of drugs is avoided.

Others- inhibition of COMT as described above is a useful therapeutic alternative to many PD patients. However, there are associated side effects and toxicities associated with this drug family. In some patients there is a clinical liver enzyme elevation that requires monthly monitoring and liver function tests are routinely administered every 6 weeks for the first three months of therapy. Liver impairment can result in the reduction of drug detoxification mechanisms, and clinically as jaundice.

Because COMT and monoamine oxidase are the two predominant metabolizing enzymes for catecholamines, concurrent therapy of a COMT and a non-selective monoamine oxidase inhibitor may result in aberrant neuroexcitotoxicity. However, selective monoamine oxidase inhibitors of MAO-B may be administered together.

Other side effects include dyskinesias, nausea, sleep disorders, dystonia, excessive dreaming, anorexia, muscle cramps, and orthostatic hypotension.

Surgical treatment and implantable devices- both pallidotomy and thalamotomy are routinely considered for the treatment of refractory essential tremor. The extent and level of surgical success is dependent on accurate localization of the globus pallidus or the thalamus. Surgery that includes either of these two methods is a one attempt procedure, too much surrounding brain tissue may be lost in subsequent procedures. A side effect may be loss of cerebral function in surrounding areas that may or not result in clinical relevant or observable disease.

Impact of Genotyping on Drug Development for PD

For Parkinson's disease, there is evidence to suggest that there are efficacy and safety differences to drug therapy in the PD patient population. Although not all of these responses may be attributable to genotypic differences, it is expected that if stratification based upon genotype were performed, a reasonable correlation between drug response and genotype may become obvious. As described below, there are gene pathways that are involved with current drug therapy and those that may be potentially involved in the future. As described in the Detailed Description, methods for the identification of candidate genes and gene pathways, stratification, clinical trial design, and implementation of genotyping for appropriate medical management of a given disease is easily translated for PD. As described below in section V. below there are likely gene pathways as are those that are outlined in the gene pathway table 2 and the matrix table 7.

Description of Mechanism of Action Hypotheses for Future Drug Development

Motor symptoms of PD result primarily from the degeneration of dopaminergic innervation within the putamen and the caudate nucleus. Further dopaminergic degeneration within the mesocortical and mesolimbic systems may be responsible for the cognitive deficits and neurobehavioral symptoms. Autonomic dysfunction often observed in PD patients may be the result of loss of dopaminergic function in the hypothalamus. Although dopaminergic pathways have been studied extensively in post mortem PD patients loss of neurotransmitter pathways that may be responsible for additional clinical symptomology. For example, loss of noradrenergic innervation in the locus ceruleus may contribute to the sudden and unpredictable freezing of motion and degeneration of cholinergic neurons in cortical areas may lead to observed dementia in PD patients.

There have been recent proposals for the mechanism of selective neuronal cell death and functional loss. The proposed mechanisms involved in the

progressive degeneration of dopaminergic neurons are oxidative stress, mitochondrial dysfunction, excitotoxic damage, cell death. Below each is described, with proposed gene targets.

Oxidative stress: In oxidative stress, generation of reactive oxygen species, part of the normal cellular metabolism, is aberrant and levels exceed the regulated cellular metabolism or scavenging mechanisms. The free radicals are generated by the conversion of superoxide ions to hydrogen peroxide via the enzyme superoxide dismutase and the reaction of hydrogen peroxide with reduced glutathione to produce water under the control of glutathione peroxidase. Since it has been documented a 60% reduction in the available reduced glutathione as well as an increased generation of iron associated with neuromelanin, there is a potential shift in the balance of the capacity to scavenge hydrogen peroxide radicals.

Oxidative stress may also be part of circuitous pathway leading to cell death that is as follows: generated free radicals lead to mitochondrial damage, which leads to neuron excitotoxicity, which leads to increased concentrations of intracellular calcium which increases the generation of free radicals. All four pathways (free radicals, mitochondrial damage, neural excitotoxicity, and increased intracellular calcium) can independently lead to neuron cell death. Neuroprotective agents, antioxidative agents, and those agents having effects of halting, retarding, or preventing progression of neurodegeneration may affect one or more of these pathways leading to therapeutically relevant agents.

Mitochondrial damage: In mitochondrial damage, the evidence is born out of the experiments of the specific neurotoxin, MPTP. MPTP is a protoxin, its active form MPP⁺ has been shown to result from its inhibition of mitochondrial respiration at the level of complex I, the complex that controls the transfer of one electron from NADH to co-enzyme Q and the transfer of two protons to the mitochondrial inner space, both are then used to synthesize ATP from ADP. In addition, MPP⁺ is thought to increase leakage of electrons at complex I, thereby increasing the generation of superoxide. Since the association of MPTP and the evolution of PD in intravenous drug users, it has been shown that there is a decrease in complex I activity in the substantia nigra in PD patients and is relatively unique to PD than other neurodegenerative disorders.

Excitotoxic damage: In excitotoxic damage, the theory posits there is an excess glutaminergic signal from the neocortex and the subthalamic nucleus to the substantia nigra. The excess signal, by acting at NMDA receptors, changes the

permeability of the neural cells to calcium which leads to aberrant post synaptic membrane potentials, enhanced propensity for depolarization and latent repolarization, and activation of nitric oxide synthase (NOS). Activation of NOS leads to the generation of free oxygen radicals through the peroxynitrite reaction.

5 Since the discovery that output neurons of the subthalamic nucleus provide a glutaminergic excitatory input to the substantia nigra, increased calcium influx into the cells and increased formation of nitric oxide via the activation of NOS, may be particularly harmful in PD due to the defect in mitochondrial complex I (see above). Excitotoxic damage to the substantia nigra, thus potentially stems from the integrity
10 of the substantia nigra and or overactivity of the subthalamic nucleus. Thus, strategies aimed at dual actions of enhancing dopaminergic status (dopamine agonism) in the substantia nigra and reducing subthalamic overactivity (glutaminergic antagonism).

Cell Death: In neural cell death, neurons in the substantia nigra undergo death
15 signals via necrosis and apoptosis. In studies involving double labeling with the TUNEL assay (apoptosis) to determine DNA fragmentation and cyanine dye labeling to determine cell structural detail, it was shown that DNA fragmentation and chromatin condensation occurs in the same nuclei of neurons in substantia nigra in patients with PD. Therefore, it appears that the number of apoptotic nuclei in the
20 substantia nigra in PD is greater than that seen in normal aging, consistent with the 10-fold higher rate of cell loss observed in patients with PD. Thus, antiapoptotic agents or therapies may halt, retard, or prevent the progression of neurodegeneration.

Neuroprotection afforded by growth factors in general or specific to neurons
25 have been considered. Growth factors including but not limited to BDNF, GDNF, bFGF have been studied in preclinical animal models of PD. Furthermore, GDNF has been tested in clinical trials.

Alternative neurotrophic agents are a group of ligand called the immunophilins. These ligands have been shown to have neurite growth promoting
30 and neuroprotective effects. Although these effects were first described from results of experiments of the immunosuppressive agents, cyclosporine and FK-506, nonimmunosuppressive analogues have been generated to have neuroprotective capacity while having none of the immunosuppressive qualities. These low molecular weight ligands may hold promise for the medical management of PD.

Based upon these varying hypotheses as stated above, there are many products in development for PD. Table 34 below lists current therapies that are in development for PD.

5 F. Spasticity

Description of Spasticity

Spasticity is a complication that occurs in patients with diagnosed neurodegenerative diseases or cerebral insults such as multiple sclerosis, cerebral palsy, tetanus, traumatic brain injury, post traumatic spinal cord injury, amyotrophic lateral sclerosis, dystonic syndromes (axial dystonia), and stroke. Together there are approximately 1.8 million individuals with spasticity in the U.S. Spasticity is a term that generally refers to one of a variety of forms of muscle hypertonicity, hyperactive muscle stretch reflexes, exaggerated tendon reflexes, and clonus and flexor spasms. Spasticity is commonly described as an isokinetic movement disorder distinguished by velocity-dependent increase in muscle tone characterized by hyperactive stretch reflexes. Patients with spasticity have impaired voluntary control of skeletal muscles, difficulty relaxing muscles once movement has stopped, difficulty initiating rapid movements, and an inability to regulate controlled movement.

20 Clinically, there are three types of spasticity 1) mild, characterized by hyperactive reflexes and unsustained myoclonus; 2) moderate, characterized by involuntary, uncontrolled contractions, sustained myoclonus neither of which affects activities of daily living; and 3) marked or severe, characterized by unpredictable, uncontrolled paroxysms of spasm and involuntary clonus; these can throw the patient from a wheelchair and often the patient cannot lie in bed quietly; these patients have difficulties using a wheelchair, and transfers (for example: from bed to chair) are problematic.

30 Broadly speaking there are two groups of spasticity patients: cerebral origin spasticity (etiologies resulting from congenital or acquired injuries such as trauma (traumatic brain injury), anoxia (cerebral palsy), or stroke); spinal origin spasticity (etiologies include spinal cord injury and multiple sclerosis). Uncontrolled spasticity exacerbates physical disabilities, increases the cost of care, and profoundly impacts the quality of life for the patient and family.

35 Current Therapies for Spasticity

Mild to moderate spasticity is medically managed with the available treatments. Little to no data are available with respect to waning of efficacy or

progression of the spasticity to more severe forms. With prolonged marked spasticity, contractures (static muscle shortening due to chronic spasm) may develop so that neither lying nor sitting occurs without undue pressure on bony prominences which lead to chronic pressure sores.

5 As the severity of the spasticity is a continuum, so are the therapies. Spasticity may not require treatment until it becomes painful, bothersome to the patient, or interferes with the activities of daily living. Existing treatments for spasticity may be categorized as systemic or locally acting.

10 Systemic Oral Medications

These are dantrolene (interferes with the excitation-contraction coupling mechanism by interfering with Ca^{++} (dantirum), baclofen (GABA_B agonist, lioresal), diazepam (GABA agonist, valium), tizanidine hydrochloride (β_2 -agonist, zanaflex). Back-up medication is the α -agonist, clonidine.

15

Locally Acting Treatments

Locally acting treatments include intrathecal baclofen, surgical or chemical rhizotomy, and nerve motor point blocks.

Intrathecal baclofen

20 Oral Baclofen is associated with undesirable side effects, however, Baclofen can be delivered to the subarachnoid space attached to a subcutaneous pump. Intrathecal baclofen is a convenient therapy and this form of drug delivery poses fewer central side effects. Further, intrathecal baclofen has shown to reduce spasticity, improve functional capabilities, and increases functional range of passive
25 movement.

Surgical intervention

This category includes rhizotomy, which has been most successful in the treatment of spasticity in children with cerebral palsy. In elderly patients that may have stroke induced spasticity, rhizotomy is uncommon and virtually not considered.
30 Another surgical procedure, tendon lengthening, can be considered in those patients in which the lower extremities are affected. This procedure can be considered in those stroke patients who have developed spasticity.

Chemical Rhizotomy

35 Chemodenervation is performed via injections of phenol (or ethanol) or botulinum toxin. In phenol injections, there is neurolysis of the motor nerve. This nerve block technique is useful for motor neuron associated spasticity, and is generally avoided in cases where sensory and motor neurons are hyperactive. The improvement of spasticity after phenol injections may last for a few weeks to years.

Botulinum toxin (BTX) injection into motor neurons has proven useful in the treatment of spasticity. This potent neurotoxin isolated from *Clostridium botulinum*, acts by binding to receptors at the neuromuscular junctions. The binding to the type A toxin is highly specific. The deactivation of intracellular presynaptic vesicles to release acetylcholine in the synaptic cleft can re-establish normal muscle tone and contractility. Intramuscular delivery of BTX has the advantages of lack of sensory effects, lack of caustic chemicals such as phenol, ability to target specific muscle groups through the use of electromyography, and an ability to weaken muscles in a graded fashion.

10 Limitations of Current Therapies for Spasticity: Efficacy and Toxicity
Systemic Local Medications

With the exception of dantrolene (which acts on directly on muscle), all of the other oral medications act on the central nervous system and there are unwanted effects from the medications, i.e. drowsiness and confusion. Dantrolene and baclofen may cause hepatotoxicity, and dantrolene may cause weakness in other muscle groups. Further, the systemic treatments are highly nonselective. As listed above, there are some indications that these oral medications are less likely reduce the spasticity; outcomes of oral medications in the treatment of cerebral origin spasticity are poor as compared to good outcomes in patients with spinal origin spasticity. Often combination regimens are used to attempt to curb the myoclonus.

20 Locally Acting Treatments

Intrathecal Baclofen- The limitations of this method of delivery are numerous: pump failure, infection, catheter migration, and the need to refill the reservoir. The half-life for ITB is 4-5 hours, and the pump must be refilled at least every 90 days.

Chemodenervation this technique is dependent on the proficiency of the surgeon and the accuracy of motor stimulation electromyography (EMG). Phenol injection close to a sensory nerve can result in causalgia due to injury of the myelin sheath of the sensory nerve.

30 BTX- There are studies that demonstrate a resistance to the toxin, these studies have shown that an antibody titer to the toxin prevents full potency.

Impact of Pharmacogenomics on Drug Development for Spasticity

As described above, there is evidence to suggest that there are efficacy and safety differences to drug therapy in the spasticity patient population. Although not all of these responses may be attributable to genotypic differences, it is expected that if stratification based upon genotype were performed, a reasonable correlation between drug response and genotype may become obvious. As described below, there are gene pathways that are involved with current drug therapy and those that

may be potentially involved in the future. As described in the Detailed Description, methods for the identification of candidate genes and gene pathways, stratification, clinical trial design, and implementation of genotyping for appropriate medical management of a given disease is easily translated for spasticity. As described
5 below in section V. below there are likely gene pathways as are those that are outlined in the gene pathway table, Table 2, and the gene pathway /indication matrix table, Table 7.

Optimization of GABAergic or ion channel modulation mediated therapy of spasticity further demonstrates the utility of selection of a potential spasticity patient
10 that has a predisposing genotype in which selective antispasticity or agents are more effective and or are more safe. In considering an optimization protocol, one could potentially predetermine variance or variances within the GABAergic receptor, ion channel or ion channel mediated mechanisms of neurotransmission, or GABAergic receptor mediated intracellular mechanism of action that is preeminently responsible
15 for drug response. By embarking on the previously described gene pathway approach, it is technically feasible to determine the relevant genes within such a targeted drug development program for spasticity.

A sample of therapies approved or in development for preventing or treating the progression of symptoms of spasticity currently known in the art is shown in
20 Table 36. In this table, the candidate therapeutics were sorted and listed by mechanism of action. Further, the product name, the pharmacologic mechanism of action, chemical name (if specified), and the indication is listed as well.

Description of Mechanism of Action Hypotheses for Future Drug Development for Spasticity

25 Although the exact mechanism of neurodegeneration-induced spasticity is unknown, the pathophysiology centers on the inadequate release of the inhibitory neurotransmitter, GABA within the spinal cord. Cerebral damage or localized damage within the spinal cord can influence the descending neurons that normally release GABA. However, the afferent input to the spinal cord from the muscle
30 spindles is unaffected causing a relative increase of excitatory neurotransmitters, particularly glutamate. The consequence is excessive stimulation of the alpha motor neurons resulting in spasticity. Spasticity arising from cerebral damage may only affect certain modulatory inhibitory signals resulting in a variability of spasticity within each and among patients. Since all muscle groups may not be affected
35 equally, management may be complicated.

Spastic paresis or spastic dystonia appear to arise from an imbalance of inhibition and excitation occurring at the level of the motor neuron. The most basic

component is the abnormal intraspinal response to sensory input. Since modulation of the local spinal cord activity (peripheral segmental reflex arcs and the anterior horn cells) occurs via the descending pathways, loss of the GABA interneurons can affect the balance of excitation/inhibition and leads to hyperexcitable cells that result in an increase in activity of by the extrafusal muscle fibers.

Further, there may be genes within pathways that are either involved in metabolism of neurotransmitters or are involved in metabolism of various drugs or compounds. In Tables 2, 13, and 19, there are listings of candidate genes and specific single nucleotide polymorphisms that may be critical for the identification and stratification of a patient population diagnosed with spasticity based upon genotype. Current pathways that may have involvement in the therapeutic benefit of epilepsy include glutaminergic, adrenergic, cholinergic, GABAergic, calcium channel, mitochondrial maintenance, adhesion, and myelination gene pathways that are listed in Tables 2, 13, and 19. One skilled in the art would be able to identify these pathway specific gene or genes that may be involved in the manifestation of spasticity, are likely candidate targets for novel therapeutic approaches, or are involved in mediating patient population differences in drug response to therapies for spasticity.

G. Ischemic Cerebrovascular Disease

Description of Stroke

Ischemic cerebrovascular disease is a result of an imbalance of the oxygen supply and the oxygen demand of brain tissue. Stroke is a series of clinical manifestations of reduction of blood supply to the cerebrovascular bed. The signs and symptoms may be complex and depend on the location and extent of the infarct.

Ischemic cerebrovascular disease is divided into thrombotic and hemorrhagic stroke.

Thrombotic Strokes

Strokes are the result of reduced blood flow supplied by one or more of the major cerebral arteries. Blockage or reduction of blood volume to these main arteries manifests as identifiable neurological symptoms. For example, occlusion of the middle cerebral artery results in contralateral hemiparesis, expressive aphasia, anosognosia and spatial disorientation, contralateral inferior quadrantanopsia, contralateral hemiparesis, sensory loss, contralateral homonymous hemianopsia, or superior quadrantanopsia. Blockage or reduction of the inner carotid artery, anterior cerebral artery, vertebral or basilar arteries, or the posterior artery can result in similarly clinically distinct neurological symptoms.

Transient ischemic attacks (TIA) are similar to a thrombotic stroke in that neurological deficit lasts for a brief period and is generally treated with potent platelet aggregation inhibitors.

Thrombotic strokes are the result of focal blockage of one or more of the cerebral arteries or branches resulting in neurological signs and symptoms lasting greater than one hour. Artherosclerotic plaques in extracranial or intracranial arteries cause approximately two thirds of thrombotic strokes. Embolization, stenosis, or occlusion of one or more of the cerebral arteries or branches may cause thrombotic strokes. Emboli can be of cardiac origin (e.g. mural thrombi, valvular heart disease, arrhythmias (atrial fibrillation), cardiac myxoma, and paradoxical emboli (venous origin). Focal ischemia may also be the result of inflammation and necrosis of extracranial or intracranial blood vessels, i.e. vasculitides (e.g. primary cerebral arteritis, giant cell vasculitis, infectious vasculitis) or the result of hematologic abnormalities (hemoglobinopathy, hyperviscosity syndrome, hypercoagulable states, protein C or S deficiency, the presence of antiphospholipid antibodies). Strokes may be drug related, for example illicit drugs (cocaine, "crack", amphetamines, lysergic acid, phencyclidine, methylphenidate, sympathomimetics, heroin, and pentazocine), ethanol, and oral contraceptives. Lastly there are other diseases that may predispose an individual to a stroke, for example fibromuscular dysplasia, arterial dissection, homocystinuria, migraine, subarachnoid hemorrhage, vasospasm, emboli of other origin (fat, bone, and air), and moyamoya.

Hemorrhagic Strokes

Approximately 20% of all strokes are the result of intracranial hemorrhage. Approximately half of these cases are into the subarachnoid space and the other half directly in the cerebral tissue. The acute rise in intracerebral pressure generally results in loss of consciousness and many die of cerebral herniation. Similar to thrombotic strokes, hemorrhagic strokes can be considered diffuse or focal, depending on the extent of the vessel disruption. Causes of spontaneous intracranial hemorrhage include arterial aneurysms (berry aneurysms, fusiform aneurysm, mycotic aneurysm, and aneurysm with vasculitis), cerebrovascular malformations, hypertensive-atherosclerotic hemorrhage, hemorrhage into a brain tumor, systemic bleeding diatheses, hemorrhage with vasculopathies, hemorrhage with intracranial venous infarction. Subarachnoid hemorrhage is caused by rupture of surface arteries (aneurysms, vascular formations, head trauma) with blood limited to the cerebrospinal fluid space between the pial and the arachnoid membranes.

Current Therapies for Stroke

If a hemorrhagic stroke is clear on the CCT, gradual reduction of systemic BP is achieved by standard vascular dilatation medications. Angiography can be useful to identify the source of the hemorrhage. Surgical management of the hemorrhage may be required.

If an ischemic stroke is identified and focal neurological impairments subside over time, a transient ischemic attack (TIA) is suspected. TIA has a high rate of recurrent stroke within a short time frame. Platelet aggregation inhibition is standard therapy; aspirin or ticlopidine. Ticlopidine is associated with neutropenia and agranulocytosis which may be life threatening. Because of these severe side effects, Ticlopidine is reserved for patients who are intolerant to aspirin therapy.

If angiographic review a clearly defined clot is detected, TIA may be surgically treated with endarterectomy.

For the treatment of thrombotic or embolic strokes, each case is independently assessed for surgical management or anticoagulant therapy. The success of thrombotic therapy, e.g. tissue plasminogen activator (tPA), streptokinase, urokinase, relies on timely reperfusion. The therapeutic window for tPA has been shown to be within three hours of onset of symptoms. Hypothermia has been shown to decrease mortality and improve outcomes. Hyperthermia has been shown to worsen both mortality rates and outcomes.

Significant neurologic improvement has been shown to occur within the first three months after stroke symptoms. A clear focus on intensive rehabilitation during this critical time frame has been shown to enhance the eventual outcome for survivors of stroke.

Limitations of Current Therapies for Stroke

The single most limiting factor of stroke therapy is the rapid identification of stroke symptoms and urgency of intervention within a short time.

Limitations of Stroke Therapy Due to Low Efficacy and Deleterious Side Effects

Guidelines for the use of tPA in acute ischemic stroke call for the administration of the thrombolytic agents within the first three hours from the onset of symptoms. After three hours four probable deleterious effects have been proven in animal studies and are as follows: 1) cerebral and extracerebral hemorrhage, 2) reperfusion injury, 3) fragmentation of clots, and 4) reocclusion of reperfused vessels.

In both animal models and in humans, reperfusion therapy must be administered within three hours of symptom onset. After three hours deleterious reperfusion injury may occur. Mortality at three months was 17% in the tPA group and 21% in the placebo group ($p=0.30$). Tissue plasminogen activator (tPA), streptokinase, heparin, and urokinase have specific restrictions: tPA has a 6% rate of cerebral hemorrhage; streptokinase is generally not used for thrombotic strokes because of serious side effects and limited quantifiable efficacy, urokinase is generally delivered near the site of the clot or obstruction. Factors influencing the best medical treatment of ischemic stroke must weigh the benefits and limitations of each of these therapies.

Impact of Genotyping on Drug Development for Stroke

As described above, there is evidence to suggest that there are efficacy and safety differences to drug therapy in the stroke patient population. Although not all of these responses may be attributable to genotypic differences, it is expected that if stratification based upon genotype were performed, a reasonable correlation between drug response and genotype may become obvious. As described below, there are gene pathways that are involved with current drug therapy and those that may be potentially involved in the future. As described in the Detailed Description, methods for the identification of candidate genes and gene pathways, stratification, clinical trial design, and implementation of genotyping for appropriate medical management of a given disease is easily translated for stroke patients. As described below in section V. below there are likely gene pathways as are those that are outlined in the gene pathway Table 2 and matrix Table 7.

A sample of therapies approved or in development for preventing or treating the progression of symptoms of stroke currently known in the art is shown in Table 37. In this table, the candidate therapeutics were sorted and listed by mechanism of action. Further, the product name, the pharmacologic mechanism of action, chemical name (if specified), and the indication is listed as well.

Mechanism of Action Hypotheses for Novel Therapies for Stroke: Utility of Genotyping

There are two categories of genotyping that provided insight on the selection of candidate genes for polymorphic genotypic studies of drug response. One set of likely candidates come from disease etiology or linkage studies. These data may provide input on the genetic etiology or aberrant mechanisms of strokes. Another set are those genes involved in the biochemical or molecular mechanisms of drugs, agents, or candidate therapeutic interventions.

Genes Involved in the Etiology of Stroke

Studies have demonstrated that there is a genetic component to thrombotic stroke. These genetic factors may predispose by an individual to thrombotic stroke by inheriting one or more of the following 1) low threshold for aberrant formation of atherosclerotic plaques in intracranial blood vessels; 2) traits that underlie certain specific etiology of stroke; and 3) a disease, disorder, or pathophysiologic process of the CNS in which there are associated molecular or structural disturbances that predispose individuals to strokes. These genetic influences mediating stroke may be candidates for genotyping assays and directly linked to pharmacogenomic programs.

Genes Involved in the Mechanism of Drug Action

There are also the biochemical, or molecular mechanisms of drug or candidate therapeutic action that may affect drug action. As described above there is an urgent need for the discovery and development of therapeutic alternatives for the medical management of strokes in which therapy commences beyond the therapeutic windows of thrombolytics.

Recent research and development programs have included the following pathways: 1) glutamate neurotransmitter pathway has been implicated in aberrant excitatory neurotransmission; 2) inflammation is a mechanism that may lead to profound neural cell loss, 3) carnosine pathway, 4) cell adhesion pathways, 5) oxidative stress pathways, 6) growth factor mediated differentiation and rescue of ischemic tissue, and protein maturation and degradation.

Ischemic Penumbra, Site of Infarct-Tissue at Risk

Ischemic penumbra is the tissue immediately adjacent to the infarct zone that is viable and morphologically intact but functionally impaired due to the restricted blood flow. Once the blood flow decreases to a certain threshold, this penumbra tissue can be classified as "misery-perfused" because oxygen consumption is preserved and increased oxygen extraction occurs. Ischemic penumbra is, thus, a dynamic process of impaired perfusion and unstable energy metabolism. Since necrosis naturally follows the continued oxygen deprivation, it has been reported that final cerebral infarct size is infarct zone plus the unrecoverable penumbra.

Functional imaging of the cerebral infarct can detect the penumbra tissue, and in some reports the penumbra tissue can be identified up to 48 hours. There is controversy whether the penumbra tissue can be rescued and what is the appropriate time from symptom onset to rescue by reperfusion. Rescue and time to rescue by reperfusion is dependent on the extent of occlusion and severity of metabolic disturbances. Based upon the hypothesis that early, immediate reperfusion can

restore blood flow, the therapeutic window for successful intervention to restore the metabolic alterations has been postulated and proven to be within the first three hours from symptom onset. Other therapies include restoration of the cytokine, neurotransmitter, and Ca^{+2} concentrations within the infarct zone (see therapy for stroke below).

Since the therapeutic window for victims of stroke is narrow and the debilitating effects of an ischemic stroke can be both costly and severely impact health-related quality of life, there is demand for candidate therapeutic interventions that can halt, retard, prevent neural destruction. Furthermore, there is a demand to develop further candidate therapeutic interventions that can assist in the rehabilitation and ultimately improve the health-related quality of life indices.

Inflammation and Immune Disease, Disorders, or Dysfunctions

Exemplary diseases characterized by abnormal inflammatory or immunologic responses (also referred to herein as inflammatory or immune diseases or disorders) are described below. These diseases are suitable for application of the methods described in this invention for identification of variances in a gene or genes involved in therapeutic response, e.g. efficacy, tolerability or toxicity.

A. Arthritis

Description of Arthritis

Arthritis comprises a variety of diseases characterized by pain, swelling, and limited movement in joints and connective tissues. Arthritis is usually chronic and there are three prevalent forms of the disease: rheumatoid arthritis (RA), osteoarthritis (OA), and fibromyalgia. In RA, the synovial joint lining becomes inflamed as a result of hyperactive immune response. There are an estimated 2.1 million Americans with RA; two thirds are women. In OA, the cartilage that covers the ends of the bones within joints deteriorates, causing pain and loss of movement as bone begins to rub against bone. There are an estimated 20.7 million Americans with OA, the majority being over the age of 45. In fibromyalgia, widespread pain affects muscles, attachments of muscles to bone, and the connective tissues, i.e., the ligaments and tendons. There are an estimated 3.7 million individuals diagnosed with fibromyalgia syndrome. Other serious and common forms of arthritis or related disorders include the following: gout, systemic lupus erythematosis, scleroderma, ankylosing spondylitis, and juvenile arthritis.

Rheumatoid arthritis involves the disarthroidal joints and can affect a variety of other organs. The clinical hallmarks of RA include: morning stiffness; swelling of three or more joints; swelling of hand joints (proximal interphalangeal, metacarpophalangeal, or wrist); symmetric swelling; subcutaneous nodules; serum

rheumatoid factor; and erosions and or periarticular osteopenia, in hand or wrist joints, often observed on radiograph.

Osteoarthritis is a degenerative process in joint tissues that may occur in response to aging, genetic, and environmental factors. It is characterized by progressive degeneration of cartilage, bone remodeling, and overgrowth of bone. The clinical hallmarks of OA include: deep aching pain in the afflicted joints (hands, knees spine, and hips), morning stiffness of short duration, variable joint thickening and effusion. Pathologically OA is characterized by breakdown of cartilage. Destruction of joint cartilage involves direct physical injury, enzymatic degradation as a result of the injury to chondrocytes, and subchondral bone stiffening as a result of the bone remodeling.

Current Therapies for Arthritis

Agents used to treat RA fall into one of the following four categories: analgesics (NSAIDs, salicylates), disease modifying antirheumatic agents (gold compounds, cytotoxic), hormones (glucocorticoids), and skin and mucosal membrane preparations. Therapies for the treatment of OA focus on decreasing pain (analgesics) and physical therapies to increase joint mobility.

Analgesics: Typically, pain associated with arthritis can be controlled with NSAIDs including but not excluded to, salicylates, para-aminophenol derivatives, indole and indene derivatives, heteroaryl acetic acids, arylpropionic acids, anthranilic acids, enolic acids, or alkanones. Antiinflammatory agents such as cyclooxygenase inhibitors, lipoxxygenase inhibitors, and others can be used to block the inflammation physiological pathway which mediate pain and the progression of the disease. However, because these drugs are limited in their efficacy in advanced or more severe stages of arthritis, these agents are add-on therapies.

NSAIDs derive their principle mechanism of action by the inhibition of prostaglandin and leukotriene synthesis. These compounds inhibit key enzymes in the biosynthetic pathway, i.e. cyclooxygenase. There are drugs that selectively inhibit isoforms of cyclooxygenase 1 and 2 (COX-1, COX-2) which enhances patient tolerance due to the prevalence of COX-2 induction occurs in inflammation mediated by cytokines and others.

Further, pyrimidine synthesis inhibitors can be used as an antiinflammatory agent in arthritis, e.g. leflunomide.

Disease-Modifying Antirheumatic Drugs or agents: Agents involved in the modification of clinical disease manifestation, reduction in inflammation, or slow the progression of the disease are referred to as disease-modifying antirheumatic drugs (DMARDs) and include gold salts (aurothioglucose, aurothiomalate,

auranofin), hypotensives (angiotension converting enzyme inhibitors), anaprox, immunosuppressives (azathioprine, cyclosporine), agents to treat metallic poison (penicillamine), depen, naprosen, immuran, antimalarials (chloroquine, hydroxychloroquine), alkylating agents (cyclophosphamide), absorbable
5 sulfonamides (sulfasalazine), irritants and counter-irritants (capsaicin), antimicrobial agents (tetracyclines), and antimetabolites (methotrexate).

Hormones and Growth Factors: Agents acting at hormone receptors or growth factor receptors include steroids (glucocorticoids), adrenocorticotrophic hormone (corticotropin), and tumor necrosis factor inhibitors (soluble TNF receptors
10 (enbrel) and TNF monoclonal antibody (remicade). Since the autoimmunity component of the disease is driven primarily by activated T-cells, which give rise to cytokines IL-1 and TNF at the rheumatoid synovium. These agents are known to interfere with the actions of these cytokines.

Corticosteroids affect the inflammation within the joints by decreasing
15 growth and development of mast cells, inducing apoptosis, suppressing lymphocyte generation of IL-5 and other cytokines, inhibiting some mediator release, inhibiting cytokine production, inhibiting the transcription of cytokines (for example IL-8, TNF- α , prototypic antiviral chemokine (regulated-on-activation normal T-expressed and secreted, RANTES), and GM-CSF), and inhibiting nitric oxide synthesis.

Skin and mucosal membrane preparations: irritants and counter-irritants can
20 be used to treat arthritic joints and include, but not limited to, Capsaicin

Chlorambucil, cyclosporine, cyclophosphamide are agents that are available for use in the treatment of refractory RA or with severe extraarticular complications such as vasculitits, corneal perforation or other severe systemic maladies associated
25 with RA.

Low Efficacy Limitations of Therapies for Arthritis

The therapies discussed above are limited to the slowing or retarding the progression of arthritis. As degeneration of the joints progresses, and irreversible
30 damage occurs, the options become limited. Thus, therapies for arthritis are aimed at reduction of manifestation of symptoms by controlling the clinical manifestations of inflammation.

The reduction of clinical symptoms of arthritis following DMARDs therapy is only evident after several weeks to months after therapy. The slow clinical
35 relevance of these therapies limits the clinician to determine optimal therapy for individuals with arthritis, and provides a risk for selection of optimal therapy for any given stage of the disease.

Toxicity or Undesired Side Effects as Therapeutic Limitations of Arthritis

There are toxicities and undesired side effects associated with the above current therapies for arthritis that require monitoring. Drugs used to treat arthritis may cause death, disability, disease, and place an unborn child at risk. The undesired side effects or toxicities are listed for each drug category as described above.

Analgesics associated side effects include dyspepsia, gastric or small bowel bleeding, ulceration, renal insufficiency, confusion, rash, headache, hepatic toxicity. NSAIDs also reversibly inhibit platelet aggregation and prolong bleeding time.

Antirheumatic agents (DMARDs) associated side effects include antimalarials: retinal or macular damage; sulfonamides: hematologic toxicities (leukopenia, thrombocytopenia, hemolysis in patients with glucose 6-phosphate dehydrogenase (G6PD) deficiency); antimetabolites: hepatic compromise including hepatic fibrosis, ascites, esophageal varices, cirrhosis, pneumonitis, myelosuppression; immunosuppressives: myelosuppression, (cyclosporine: renal insufficiency anemia, hypertension); agents to treat metallic poison: rash, stomatitis, dysgeusia or metallic taste, myelosuppression (thrombocytopenia), proteinuria, nephrotic syndrome or renal failure, and induction of autoimmune syndromes (systemic lupus erythematosus, myasthenia gravis, polymyositis, Goodpasture's syndrome), gold preparations: hematologic, renal, pulmonary, and proteinuria; chlorambucil: myelosuppression, myeloproliferative disorders, malignancy, hemorrhagic cystitis.

Soluble TNF receptors agents have been shown to induce sepsis and predispose patients to serious infections. Further this product was associated with site of injection reactions, infections, and headache.

Glucocorticoid associated side effects include increased appetite, weight gain, fluid retention, acne, ecchymosis, development of cushoid facies, hypertension, hyperkalemia, diabetes, hyperglycemia, hyperosmolar state, hyperlipidemia, hepatic steatosis, atherosclerosis, myopathy, aseptic necrosis, osteoporosis, ulcers, pancreatitis, pseudotumor cerebri, psychosis, glaucoma, cataract formation, vascular necrosis, increased susceptibility to infection, impairment of the hypothalamus-pituitary axis, decreased thyroid hormone serum binding proteins, and impaired wound healing.

Since the majority of RA patients are women in their reproductive years, the level and extent the agents used to treat RA affects or has a potential to affect the mother during pregnancy, cross the placenta, affect the developing fetus, or be excreted in breast milk during lactation are important issues facing the skilled practitioner. Clinical medical therapeutic decisions must weigh the use of all of the

above current therapies for RA against known capacity of these agents to affect both the mother and the child.

5 Description of Mechanism of Action Hypotheses for Future Drug Development for Arthritis

Rheumatoid arthritis has been thought to be the result of host genetic factors, immunoregulatory abnormalities and autoimmunity, and triggering or persistent microbial infection.

10 Host genetic factors: the HLA-DR4 antigen (HLA, human leukocyte antigen) is significantly increased in RA patients. Recent studies have determined that a subtype of the HLA-DR4 share similar epitope among several MHC class II molecules and predispose to RA.

15 Autoimmune component: in over 80% of RA patients autoantibodies to the Fc portion of IgG (rheumatoid factors, RF) are present and can be used to determine diagnosis. The higher the titer of RFs the more severe joint disease and extrarticular manifestations.

Related to the autoimmune component of the disease, ICAM-1 inhibitors, or other agents to reduce adhesion have been developed.

20 Microbial Infections: of all the examined pathogens, only the Epstein-Barr virus (EBV) has remained unproven as a cause of RA. EBV has been shown to share a similar epitope as the HLA-DR4 epitopes, but EBV is ubiquitous and has yet to be a proven cause of RA.

25 A gene, genes, or gene pathway involved in the etiology of arthritis or associated disorders or potential sites for targeted drug therapy of arthritis are depicted in Table 9 with the specific gene list in Table 4. Current candidate therapeutic interventions in development for the treatment of arthritis are listed in Table 38.

30 B. Chronic Obstructive Pulmonary Disease

Description of Chronic Obstructive Pulmonary Disease

35 Chronic obstructive pulmonary disease (COPD) is an imperfect term that refers to four pulmonary disorders including simple chronic bronchitis, asthmatic bronchitis, chronic obstructive bronchitis, and emphysema. A common characteristic of the disease is airway obstruction. Airways obstruction denotes the slowing of forced expiration. A decrease in the forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) indicates that airflow is impaired. Forced expiration is determined primarily by intrinsic resistance of the airways, compressibility of the airways, and lung elastic recoil. Reduced maximal expiratory

flow results from high airway resistance, reduced lung recoil, or excessive airways collapsibility. The overall cost of these illnesses to society is enormous due to the extent of the number of individuals afflicted with COPD, approximately 15 million Americans, and that COPD is currently the fourth-leading cause of mortality. The high morbidity and mortality rates associated with COPD are linked to the failure to identify at-risk patients and intervene. The lungs have large reserves of pulmonary function and the slow progressive nature of the disease can often delay the clinical diagnosis and therapeutic intervention.

Simple chronic bronchitis is a syndrome predominantly characterized by chronic productive cough and is usually the result of low-grade exposure to bronchial irritants. This syndrome is associated with enhanced mucous secretion, reduced ciliary activity, and impaired resistance to bronchial infection. Bronchitis patients range from those who experience sporadic cough producing mucous to those with a severe, disabling condition manifested by one or more of the following: increased resistance to airflow, hypoxia, hypercapnia, and irreversible narrowing of the small airways, i.e. bronchioles and bronchi (2 mm or less in diameter).

Repeated exposure to bronchiole irritants in individuals with hyperactive or sensitive airways can lead to bronchospasm, i.e. bronchial smooth muscle constriction, that is frequently accompanied by excess mucous production and edema of the bronchial walls. Episodic bronchospasm in individuals with chronic bronchitis is termed asthmatic bronchitis and is applied to those individuals with chronic airway constriction, chronic productive cough, and episodic bronchospasm.

Emphysema is characterized by abnormal, excessive, permanent enlargement of airway spaces distal to the terminal bronchioles, and is accompanied by destruction of their walls and may or may not be associated with fibrotic tissue. These changes result in a reduction of elastic recoil permitting excessive airway collapse upon expiration and leads to irreversible airway flow obstruction. Emphysema is strongly related to and correlated to inhalation of tobacco smoke, i.e. cigarette or cigar smoking.

In emphysema there is a loss of elastic recoil leading to pulmonary hyperinflation. The hyperinflation reaches a limit when the diaphragm is pushed flat and no longer functions effectively. The chest wall is expanded to the point that it pushes inward rather than exerting its normal outward force. These anatomical changes alter inspiration to the point that exertion is nearly impossible.

A deficiency in alpha 1- antitrypsin can predispose individuals to signs and symptoms of COPD. In these individuals there is a marked alveolar wall destruction with a non-uniform pattern of air space enlargement. In these patients there may be excessive formation of thick mucous and is often accompanied by persistent cough.

Complications of COPD include hypoxemia, cor pulmonale, hypercapnia, and dyspnea. Sustained chronic hypoxemia is a condition that leads to pulmonary vasoconstriction that with time becomes irreversible and leads to cor pulmonale.

5 Current therapies for COPD

The current therapies is use for the treatment of subjects with COPD are aimed at reducing the airway obstruction that is reversible, controlling the persistent cough and sputum production, reducing or eliminate airway infections, increasing exercise tolerance to the maximum allowable at the individual's level of
10 physiological deficit, controlling the remedial disease complications, i.e. cardiovascular dysfunction and arterial hypoxemia, and relief of the anxiety and depression or other psychiatric symptoms that accompany patients attempts to cope with the debilitating clinical manifestations. Lastly, all treatment regimens include education and supportive therapy to encourage subjects with COPD to cease
15 behaviors that may exacerbate symptoms such as inhalation of pulmonary irritants, i.e. smoking and others, and substance abuse, i.e. narcotics and sedatives.

Bronchodilators

 Bronchodilators can be inhaled, or by oral, subcutaneous, or intravenous routes.

20 Beta-adrenergic agonists or other sympathomimetic agents are used to produce rapid acute bronchodilation.

 Anticholinergics agents are used to produce sustained bronchodilation. Nebulized atropine has been supplanted with the advent of a quaternary ammonium salt, ipratropium bromide, which undergoes minimal systemic absorption and thus
25 has limited anticholinergic toxicity. Ipratropium has been shown to be effective in patients that have not responded to β -adrenergic agonists and can reduce sputum volume without altering viscosity.

 Anticholinergics and beta-adrenergic agonist combinations have been used with some success. Such combinations reduce the need to administer high doses,
30 due to additive effects, and therefore reduce the likelihood for adverse effects or toxic side effects.

 Theophylline is a methylxanthine bronchodilator. Theophylline improves airway flow, decreases dyspnea, reduces pulmonary arterial pressure, increases arterial oxygen tension, improves diaphragmatic strength and endurance, increases
35 right ventricular function (pulmonary vasodilator and cardiac inotropic effects), and may produce antiinflammatory effects.

Expectorants

Expectorants can be used to increase the secretion clearance in patients with COPD. Although this therapy has not been demonstrated to render clinical benefit, it is as add on therapy that enables the patient to experience an enhanced productive cough.

5 Anti-Inflammatory agents

Prolonged use of corticosteroids have been used to retard the rate of decline in FEV1 in COPD subjects. However, it has been determined that systemic corticosteroids are beneficial for acute exacerbations of COPD but are not used for long-term treatment and have not been proven to retard the progression of the
10 disease. Corticosteroids affect the decline of FEV1 in the airways by decreasing growth and development of mast cells, inducing apoptosis, suppressing lymphocyte generation of IL-5 and other cytokines, inhibiting some mediator release, inhibiting cytokine production, inhibiting the transcription of cytokines (for example IL-8, TNF- α , prototypic antiviral chemokine (regulated-on-activation normal T-expressed
15 and secreted, RANTES), and GM-CSF), and inhibiting nitric oxide synthesis.

Antiproteases and antioxidants

Alpha1-protease inhibitor deficiency as a cause of early development of emphysema has increased the awareness of the role of protease-antiprotease and oxidant-antioxidant imbalances in COPD. Intravenous delivery of alpha 1-protease
20 inhibitor can provide the appropriate levels in those individuals with a genetic deficiency and those whose deficiency is acquired.

Mucolytics and secretion clearance agents can be used to assist in the removal of secretions during productive cough. These agents can thin secretions in patients with chronic bronchitis.

25 Supplemental oxygen therapy is used to treat the deleterious effects of sustained chronic hypoxemia and hypercapnia. Correction of this condition is one of the treatments shown to have a positive effect on the survival rate in patients with COPD.

 Treatment of cases of cor pulmonale includes the use of diuretics and
30 positive inotropic agents such as digitalis. Careful monitoring is required in these patients due to a development of marked right ventricular hypertrophy.

 Dyspnea may be severely disabling despite aggressive therapy. Judicious use of opiates to control dyspnea and increase exercise tolerance have been proven to be beneficial. Unfortunately, opiates can have a respiratory depressant effect and
35 care must be taken to deliver the appropriate therapeutic dose.

 Many patients with COPD find themselves anxious or depressed or both. Appropriate use of psychoactive agents can be used to control the signs and symptoms of anxiety and depression.

Surgical procedures can be performed to attempt to restore pulmonary capacity and function. Lung volume reduction surgery is useful to remove a portion of emphysematous lung tissue so that the diaphragm can return to its normal dome shape and the chest wall can reassume its normal configuration, mechanics, and physiology. Bullectomy is a procedure in which large bullae and surrounding lung tissue are removed. This allows for the remaining tissue to expand and once again function normally. Another procedure is lung transplantation. This expensive and aggressive approach is usually reserved for younger patients, particularly those who are alpha 1-antitrypsin deficient.

Limitations of Current Therapies for COPD

The most common limitations for the use of bronchodilators is the mistaken use of inhalants and inadequate patient education.

Beta adrenergic therapy is limited by three factors: 1) the density of β_2 receptors in the airways decreases with age, 2) despite the selectivity of the β_2 receptor agonists, there is cross reactivity to β_1 receptors and may affect the myocardium and other peripheral tissues, and 3) there is β -adrenergic receptor desensitization. Most of the recommended doses of beta adrenergic agonists provide less than maximal bronchodilation. Beta-adrenergic agonists can cause tremor, reflex tachycardia, tachyphylaxis, cardiomyopathy, and other cardiac toxic effects. Tachycardia is particularly problematic in the elderly or for those individuals who are at cardiac risk. Further, β -adrenergic agonists have been shown to cause hyperkalemia. The majority of patients with COPD are current or former smokers, all of whom may have coexisting coronary artery disease, thus in the compendium of therapies it is desirable to have alternatives to β -adrenergic agonists.

Anticholinergics as bronchodilators have been associated with systemic side effects. In particular, systemic anticholinergic side effects include bradycardia (if pronounced, includes compensatory tachycardia), dry mouth, inhibition of sweating, dilatation of the pupils, and visual blurring. Ipratropium has a slow onset of action and a longer duration of action than β -adrenergic agonists which can be deleterious for acute bronchodilation because patients continue to administer the drug without effect and overdose.

Theophylline continues to be a controversial treatment due to misconceptions of its role as a bronchodilator, drug delivery problems, and conflicting results of comparative studies during acute exacerbations. Further, theophylline has a limited therapeutic window, i.e. the dose required to achieve bronchodilation is close to the dose associated with undesirable or adverse side effects including convulsions,

cardiac arrhythmias, tachycardia, vasodilation, and diuresis. Further complicating therapy with theophylline is the intra-patient variability in efficacious response.

Long-term use of corticosteroids can be useful for patients in which continued symptoms or severe airflow limitations exist despite therapy with other agents. Only 20-30% of these patients experience therapeutic benefit for long-term use and indiscriminate use often leads to adverse effects without benefits.

Unfortunately there have not been identified predictors of responders or nonresponders to long term steroid use in patients with COPD. Therefore, only those patients that attempt long-term steroid use and have documented clinical improvement should continue steroid therapy. Unfortunately, those patients in which long-term steroid use results in no benefit are subjected to potential adverse effects or toxicities. Glucocorticoid associated side effects include increased appetite, weight gain, fluid retention, acne, ecchymosis, development of cushoid facies, hypertension, hyperkalemia, diabetes, hyperglycemia, hyperosmolar state, hyperlipidemia, hepatic steatosis, atherosclerosis, myopathy, aseptic necrosis, osteoporosis, ulcers, pancreatitis, pseudotumor cerebri, psychosis, glaucoma, cataract formation, vascular necrosis, increased susceptibility to infection, impairment of the hypothalamus-pituitary axis, decreased thyroid hormone serum binding proteins, and impaired wound healing.

Mucolytic and secretion clearance agents have been shown to improve thinning secretions however, there is little evidence to suggest that these agents render clinical improvement. Further cough suppressants may impair secretion clearance and possibly increase the risk of pulmonary infection.

Description of Mechanism of Action Hypotheses for Future Drug Development of Candidate Therapeutic Interventions of COPD

Since the predominant category of patients with COPD were or are current smokers smoking cessation programs and agents used to help patients quit smoking will be a valuable addition to therapeutic regimens. Nicotine replacement therapies such as nicotine patches (transdermal), gum, and transnasal formulations as well as bupropion (an antidepressant or other in this category) should be considered.

Other therapies to be considered are novel bronchodilators for inhalation therapy without the use of chlorofluorohydrocarbons (CFCs), next generation anticholinergic therapies, alpha 1 antiprotease augmentation therapies, and refinement of surgical procedures.

A gene, genes, or gene pathway involved in the etiology of COPD or associated disorders or potential sites for targeted drug therapy of COPD are depicted in Table 9 with the specific gene list in Table 4. Current candidate

therapeutic interventions in development for the treatment of COPD are listed in Table 39.

C. Autoimmune Disease

5 Description of Autoimmune disease

An immune response to "self" antigens, or autoimmunity, can vary from minimal to severe depending on the extent of the loss of self tolerance and to the localization of the antigens. There is then a distinction between autoimmune response which may or may not be pathologic and autoimmune disease which does
10 lead to pathologic conditions. In autoimmune disease there is a combination of the following types of evidence, 1) identification of the target antigens, 2) identification and isolation of self-reactive autoantibodies or self-reactive lymphocytes, 3) identification of clinical evidence, i.e. familial hereditary data, lymphocyte infiltration, MHC association and clinical symptomatic improvement with
15 immunosuppressive agents. Initiation of autoimmune disease is thought to require one or more of the following: genetic predisposition to loss of tolerance, environmental factors that stimulate aberrant immune response, or loss or dysfunction of cellular or organ physiological processes leading to pathological immune response. Since many autoreactive clones of T and B cells exist and are
20 normally regulated by homeostatic mechanisms, loss or breakdown of this system of checks and balances can lead to activation or enhancement of these autoreactive clones and ultimately lead to autoimmune disease.

There are a few autoimmune disease indications whereby inflammation and immune response gene pathways should be considered in the stratification or
25 therapeutic choice of patient groups based upon genotype. There are multiple examples of autoimmune diseases or diseases that have an autoimmune component including: amyotrophic lateral sclerosis, anti-phospholipid syndrome, aplastic anemia, autoimmune hemolytic anemia, diabetes mellitus type 1, Guillan-Barre syndrome, idiopathic thrombocytopenic purpura, Grave's disease, myasthenia
30 gravis, polymyositis, rheumatoid arthritis, Hashimoto's thyroiditis, uveitis, Wegener granulomatosis, periarteritis nodosa, ocular pemphigoid, pemphigus vulgaris, psoriasis, Goodpasture's syndrome, Churg-Strauss vasculitis, poly-dermatomyositis, Cogan syndrome- autoimmune inner ear disease, hemolytic uremic syndrome, idiopathic glomerulonephritis, inflammatory bowel disease, Crohn's disease,
35 microscopic polyarteritis, and multifocal motoneuron neuropathy. Here we discuss four specific diseases that represent larger patient populations and are representative of diseases in which therapy can be aimed at suppressing the hyperactivity of the

immune system. These include multiple sclerosis, systemic lupus erythmatosus, scleroderma, diabetes mellitus type I, sarcoidosis, and nephritis.

Multiple Sclerosis

Multiple sclerosis (MS) is a disorder of multifocal sites of myelin sheath
5 destruction, perivascular-lymphocytic cuffing and variable degree of
oligodendroglial loss. In profound cases, there is gliosis, axonal transection, and
neuronal and axonal loss. There are an estimated 300,000 Americans diagnosed
with MS. The estimated cost of MS is \$5 billion dollars.

Clinically, MS begins with a relapsing illness with episodes of neurological
10 dysfunction lasting several weeks, followed by substantial or complete
improvement. This is identified as the relapsing-remitting stage of the disease found
to be predominantly in females (1.6:1). There are some patients that remain in this
stage of the disease for decades; others may rapidly progress to the next stage. As
time progresses, and repeated relapses occur, recovery becomes less and less
15 complete or as substantial. In these cases, a gradual relapse independent clinical
progression develops and is termed secondary progressive MS. Further, the
nonrelapsing-nonremitting form is characterized by a gradual progression and steady
worsening of neurological function without any recovery or improvement. A steady
but gradual neurological decline and predominately identified in males characterizes
20 the primary progressive form of MS. Clarity in understanding the significance of
these varying disease patterns and diagnosis is dependent on quality neurological
examination overtime.

Systemic Lupus Erythmatosus

Systemic lupus erythmatosus (SLE) is a disease characterized by
25 inflammation in many different organ systems associated with the production of
antibodies to reactive to nuclear, cytoplasmic, and cell membrane antigens. Clinical
manifestations of the disease include reddish rash on the cheeks, fatigue, anemia,
rashes, sun sensitivity, alopecia, arthritis, pericarditis, pleurisy, vasculitis, nephritis,
and central nervous system disease. The immune hypereactivity appears to derive
30 from immune hypereactivity and loss of self-tolerance. In these patients antibodies
are produced against several nuclear components, notably antinuclear antibodies to
native double stranded DNA, single stranded DNA, or nucleohistones.

Scleroderma

Scleroderma is a chronic disease marked by increases of fibrotic tissue
35 involving the circulatory system, connective tissue (in particular the skin), visceral
organs, and the immune system. There are approximately 500-700,000 Americans
diagnosed with scleroderma. There are two types of scleroderma, localized and
systemic. In localized scleroderma (linear and morphea) the disorder of the

connective tissue is limited to the skin, the tissues just beneath the skin, and muscle. Internal organs are not affected. In systemic scleroderma (sclerosis) vascular, digestive, pulmonary, renal, muscle and joints may be affected. Raynaud's syndrome (frequent spasms of small arteries induced by temperature changes and emotion resulting in deprivation of blood supply to peripheral tissues), CREST syndrome (calcium deposits, Reynaud's syndrome, loss of muscular control of the esophagus, sclerodactylia, and telangiectasia), and Sjogren's syndrome (inflammation of the conductive, cornea, tear, and salivary glands with progressive destruction by lymphocytes and plasma cells) are both subcategories of scleroderma.

The clinical manifestations of scleroderma include the following symptoms: fatigue, swelling and numbness of the hands and feet, shiny skin and disappearance of skin folds, ulcers on the fingers, calcium deposits on the fingers, joint inflammation, joints tightening into bend position, muscle weakness, itchy skin, difficulty in swallowing, shortness of breath, fatty diarrhea or constipation, and loss of body hair. Although ultimately renal impairment and failure is a common endpoint, therapy affecting the hypertensive phase or renal involvement has changed the mortality rate.

Diabetes Mellitus type I

This form of diabetes involves the chronic inflammatory destruction of the insulin-producing islet cells of the pancreas. Although this form of diabetes is treated similarly to the type II form (which is not linked to autoimmunity), i.e. insulin replacement therapy, early identification of type I versus type II individuals may be useful to thwart the autoimmune destruction of the β -cells. There are an estimated 500,000 to 1 million Americans that have type I diabetes, it is the seventh leading cause of death, and the following is a list of the progressive complications that are associated with the unregulated carbohydrate balance in tissues: retinopathy leading to blindness, nephropathy (diabetic nephropathy is the leading cause of end-stage renal disease), coronary and cardiovascular disease, neuropathy (severe forms can lead to amputation), impotence (diabetic neuropathy and cardiovascular disease can lead to impotence), and stroke.

Sarcoidosis

Sarcoidosis is a granulomatous disorder characterized by enhanced cellular immune response at one or more involved sites. The prevalence of sarcoidosis is 5 in 100,000, so approximately 13,000 patients have been diagnosed. Between 80-90% of patients with sarcoidosis have pulmonary involvement, however, any organ can be affected. Pulmonary involvement includes dyspnea with or without exertion, persistent dry cough, and atypical chest pain. Cor pulmonale can develop as a complication of pulmonary dysfunction and further progress to right atria dilatation

and right ventricular hypertrophy. Ocular involvement includes disturbance in visual acuity, and in chronic cases may lead to glaucoma, cataract formation and retinal neovascularization. In 80% of the cases, sarcoidosis is self-limiting and results in minimal symptomology, discomfort, or debilitation. However in the remaining 20%, sarcoidosis patients face potentially serious debilitation, disfigurement, and can be life threatening. Misdiagnosis is frequent and can limit appropriate therapeutic intervention.

Nephritis

Inflammation of the kidneys results in impaired renal function. Nephritis can be either interstitial or glomerular. In either case, mononuclear cells infiltrate in the interstitium of the renal cortex. Eosinophils, and in some cases, polymorphonuclear leukocytes are found in a similar compartment. The infiltrate may be diffuse or patchy and may be accompanied by fibrotic tissue. Membranous nephropathy may develop and lead to impairment of glomerular filtration rate. There is evidence to suggest both cytotoxic T cells and T-cell mediate delayed hypersensitivity are involved. Nephritis is a component of the clinical manifestation of systemic lupus erythematosis, scleroderma, and other autoimmune diseases and disorders.

Current therapy for Autoimmune Diseases and Disorders

Agents used to treat autoimmune disease fall into one of the following four categories: analgesics (NSAIDs, salicylates), immunosuppressive agents, hormones (glucocorticoids), and skin and mucosal membrane preparations

Analgesics: Typically, pain associated with autoimmune disease can be controlled with NSAIDs including but not excluded to, salicylates, para-aminophenol derivatives, indole and indene derivatives, heteroaryl acetic acids, arylpropionic acids, anthranilic acids, enolic acids, or alkanones. Antiinflammatory agents such as cyclooxygenase inhibitors, lipoxygenase inhibitors, and others can be used to block the inflammation physiological pathway which mediate pain. However, because these drugs are limited in their efficacy in advanced or more severe stages of autoimmune disease, these agents are add-on therapies.

NSAIDs derive their principle mechanism of action by the inhibition of prostaglandin and leukotriene synthesis. These compounds inhibit key enzymes in the biosynthetic pathway, i.e. cyclooxygenase. There are drugs that selectively inhibit isoforms of cyclooxygenase 1 and 2 (COX-1, COX-2) which enhances patient tolerance due to the prevalence of COX-2 induction occurs in inflammation mediated by cytokines and others.

Immunosuppressive drugs or agents: Agents involved in the modification of the immune system for the treatment of autoimmune disease are immunosuppressive

agents. Immunosuppressives include azathioprine, cyclosporine, penicillamine, antimalarials (chloroquine, hydroxychloroquine), alkylating agents (cyclophosphamide), and antimetabolites (methotrexate).

5 Hormones and Growth Factors: Agents acting at hormone receptors or growth factor receptors include steroids (glucocorticoids), adrenocorticotrophic hormone (corticotropin), and tumor necrosis factor inhibitors (soluble TNF receptors (enbrel) and TNF monoclonal antibody (remicade). Since the autoimmunity component of the disease is driven primarily by activated T-cells, which give rise to cytokines IL-1 and TNF at the affected areas. These agents are known to interfere
10 with the actions of these cytokines.

Corticosteroids affect the immune response by decreasing growth and development of mast cells, inducing apoptosis, suppressing lymphocyte generation of IL-5 and other cytokines, inhibiting some mediator release, inhibiting cytokine production, inhibiting the transcription of cytokines (for example IL-8, TNF- α ,
15 prototypic antiviral chemokine (regulated-on-activation normal T-expressed and secreted, RANTES), and GM-CSF), and inhibiting nitric oxide synthesis.

Plasma Exchange: A useful technique for the removal of autoantibodies is a process called plasmaphoresis or plasma exchange. In this process, antibodies
20 are removed that mediate humoral immune response to the autoantigen.

Antioxidants: Many of the therapies in use for these autoimmune diseases are aimed at reducing the level and extent of tissue damage mediated by T-cell immune response. For example, dimethyl sulfoxide, dimethyl sulfone, para-aminobenzoic acid, and vitamin E are included in this category.

25 Limitations Current Therapies for Autoimmune Disease based upon Low efficacy

The therapies discussed above are limited to the slowing or retarding the progression of autoimmune disease. As immune response tissue damage occurs, degeneration of the function progresses, irreversible damage occurs, and therapeutic options become limited. Thus, therapies for autoimmune disease are aimed at
30 reduction of manifestation of symptoms by controlling the clinical manifestations of inflammation and the hypersensitive immune response.

 The reduction of clinical symptoms of autoimmune disease following immunosuppressive therapy by one of the agents listed above is only evident after several weeks to months after therapy. The slow clinical relevance of these
35 therapies limits the clinician to determine optimal therapy for individuals with autoimmune disease, and provides a risk for selection of optimal therapy for any given stage of the disease. Furthermore, there may be delays in identifying those

patients that have an autoimmune hyperreactivity, and this can delay therapeutic intervention.

Limitations Current Therapies for Autoimmune Disease based upon Toxicity or Undesired side effects

There are toxicities and undesired side effects associated with the above current therapies for autoimmune disease that require monitoring. Drugs used to treat autoimmune disease may cause death, disability, disease, and place an unborn child at risk. The undesired side effects or toxicities are listed for each drug category as described above.

Analgesics associated side effects include dyspepsia, gastric or small bowel bleeding, ulceration, renal insufficiency, confusion, rash, headache, hepatic toxicity. NSAIDs also reversibly inhibit platelet aggregation and prolong bleeding time.

Immunosuppressive therapies have associated side effects including antimalarials: retinal or macular damage; sulfonamides: hematologic toxicities (leukopenia, thrombocytopenia, hemolysis in patients with glucose 6-phosphate dehydrogenase (G6PD) deficiency); antimetabolites: hepatic compromise including hepatic fibrosis, ascites, esophageal varices, cirrhosis, pneumonitis, myelosuppression; immunosuppressives: myelosuppression, (cyclosporine: renal insufficiency anemia, hypertension); penicillamine: rash, stomatitis, dysgeusia or metallic taste, myelosuppression (thrombocytopenia), proteinuria, nephrotic syndrome or renal failure, and induction of autoimmune syndromes (systemic lupus erythematosus, myasthenia gravis, polymyocytis, Goodpasture's syndrome).

Glucocorticoid associated side effects include increased appetite, weight gain, fluid retention, acne, ecchymosis, development of cushoid facies, hypertension, hyperkalemia, diabetes, hyperglycemia, hyperosmolar state, hyperlipidemia, hepatic steatosis, atherosclerosis, myopathy, aseptic necrosis, osteoporosis, ulcers, pancreatitis, psuedotumor cerebri, psychosis, glaucoma, cataract formation, vascular necrosis, increased suseptibility to infection, impairment of the hypothalamus-pituitary axis, decreased thyroid hormone serum binding protiens. and impaired wound healing.

Since the majority of autoimmune disease patients are women in their reproductive years, the level and extent the agents used to treat autoimmune disease affects or has a potential to affect the mother during pregnancy, cross the placenta, affect the developing fetus, or be excreted in breast milk during lactation are important issues facing the skilled practitioner. Clinical medical therapeutic decisions must weigh the use of all of the above current therapies for autoimmune disease against known capacity of these agents to affect both the mother and the child.

Description of Mechanism of Action Hypotheses for Future Drug Development for the Treatment of Autoimmune Disease

Autoimmune disease has been thought to be the result of host genetic factors, immunoregulatory abnormalities and autoimmunity, and triggering or persistent microbial infection.

A gene, genes, or gene pathway involved in the etiology of autoimmune diseases or disorders or associated disorders or potential sites for targeted drug therapy of autoimmunity are depicted in Table 9 with the specific gene list in Table 4. Current candidate therapeutic interventions in development are listed for the treatment of autoimmune disease or disorder, Tables 40 and 42, and for systemic lupus erythematosus, Table 41.

D. Immunosuppression- Transplantation

Description of Transplantation

There are many different conditions in which medical or surgical therapy is unable to halt, retard, or treat the underlying disease, disorder, or dysfunction. Although many refractory diseases, disorders, or dysfunctions do not lead to severe cases, there are some in which the progression leads to conditions in which the remaining therapeutic alternative is replacement of the diseased tissue with normal donated tissue by transplantation. These end stage conditions include both primary disease or complications from a disease. For example whole organ transplantation is an end-stage therapeutic alternative in the following indications, end-stage cardiomyopathy, end-stage renal disease, pulmonary disease, cirrhosis of the liver, as well as other end-stage diseases affecting whole organ function.

Besides whole, or partial organ transplantation there are programs aimed at replacing cells in specific tissues to enable or restore physiologic function. For example cellular transplantation includes, but not excluded to, grafting bone marrow cells in patients with hematopoietic or lymphocytic cancers, dopaminergic producing cells in brains of patients with Parkinson's disease, striated muscle cells in patient's with Duchenne's muscular dystrophy, myocytes or cardiomyocytes in patient's with ischemic heart disease or cardiomyopathy, and replacement of neurons or astrocytes or glial cells in neurodegenerative disease including but not excluded to Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, Huntington's disease, refractory pain, epilepsy, and stroke.

In this way, transplantation includes autografts, isografts, allografts or xenografts and can involve whole organ or cellular grafts. With the exception of autologous transplantation, all other transplantation procedures include pre- and

post-surgical immunosuppression to blunt graft rejection or graft versus host disease. Successful immunosuppression in this setting includes an appropriate balance between the need to prevent the process of graft rejection and the risk of suppressing the recipient's immune system to the extent that they become vulnerable to infection or other complications.

Transplantation is immunologically mediated. Both T cells and circulating antibodies are induced against allografts or xenografts. While the antibodies are responsible for rejection of erythrocytes, T-cells are mainly responsible for the rejection of most other type of tissue. The antigens found on grafted tissue which initiate the rapid rejection of an allograft are found on most cell membranes and are encoded by genes in the major histocompatibility complex (MHC) which are called the HLA. The structures encoded in these genes, MHC class I and class II molecules, are involved in the determining the discrimination between self and non-self. The degree of the histocompatibility between donor and recipient can be determined serologically, by genotyping, or by a mixed lymphocyte reaction. Survival of HLA nonmatched allografts is prolonged by anti-inflammatory agents, cytotoxic agents, antimetabolites, and other modalities aimed at immunosuppressing the recipient. These approaches have proven clinical success in terms of graft survival and clinical symptomology.

Rejection can occur at any time, and is either hyperacute, acute or delayed. The rate, extent, and underlying mechanism of transplantation rejection varies dramatically from individual to individual. Physiological factors include patency of blood circulation, lymphatic drainage, expression of antigens on the graft, and others that can influence the rejection rate.

In hyperacute rejection, preexisting host antibodies to antigens found on the grafted tissue mount an immune response. These antibodies activate complement, followed by platelet activation and deposition causing swelling and interstitial hemorrhage in a whole organ graft, or specific cell targeting in a cellular transplant. Cell mediated immunity is not activated in the hyperacute response.

In acute rejection, infiltration of lymphocytes and macrophages recognize the foreign antigen on the graft cells, and initiate a cascade of intragraft events that ultimately leads to host cellular and humoral mediated destruction of the grafted tissue and if unchecked will result in irreversible loss of the graft. This acute process occurs rapidly and does not in the first stages affect the vital structures of a whole organ graft, which allows for identification of the process and implementation of therapy. In many cases, an acute rejection episode can be reversed, and approximately 30-50% of whole organ graft recipients undergo one or more of these episodes in the early transplant period.

Delayed or chronic rejection occurs in a slower process than acute rejection and ultimately leads to a gradual loss of function in the grafted tissues. In chronic or delayed rejection, both cell mediated immunity and humoral immunity is activated. Chronic rejection is characterized by arteriosclerosis, in which the smooth muscle cells lining the arteries in the graft organ proliferate to create lesions and lead to fibrosis, with a result of constricting blood flow. As a result of the chronic immune rejection, there is slow and progressive destruction of the grafted organ or cells. If damage to the tissue is extensive, very little can be done to save the graft.

10 Current Immunosuppressive Therapies

The goal of clinical immunosuppression in the transplantation setting is to control allograft rejection. Clinical immunosuppression involves the non-specific suppression of both cell-mediated and humoral immune reactivity to the grafted tissue. Although a number of methods have been proposed, successful prolongation of graft survival has been attained through the use of a combination of therapies that suppress both the lymphocytic interaction and proliferation and therapies that deplete the pool of available lymphocytes.

Antiproliferative agents

These agents are useful to blunt the proliferative phase of lymphocyte activation of the immune response.

Purine analogs

Azathioprine acts to inhibit the proliferation of T cells. Azathioprine is cleaved to 6-mercaptopurine and it is this active compound that serves to suppress the T-cell mediated antigenic determination and engraftment. Azathioprine is a relatively non-selective immunosuppressive agent. Other agents in the same class as azathioprine, i.e. antimetabolites, include but are not excluded to, mercaptopurine, chlorambucil, and cyclophosphamide.

Pyrimidine analogs

The agents (cytosine arabinoside) inhibits DNA synthesis and therefore have their greatest effect on the immune response during the proliferative phase of lymphocyte activation. These agents inhibit primary antibody response and have minimal effects on the cell-mediated immunity.

Folic acid analogs

These agents (methotrexate, aminopterin) inhibit dihydrofolate reductase preventing the conversion of folic acid to tetrahydrofolic acid. This conversion is necessary for the production of DNA and RNA.

Alkylating Agents

These agents (nitrogen mustard, phenylalanine mustard, busulfan, cyclophosphamide) alter the structure of the DNA and RNA. These agents have reactive ring structures which combine with electron rich groups such as tertiary nitrogen in purines or pyrimidines, or -NH₂, -COOH, -SH, -PO₃H₂ groups. These reactions alter the composition of the DNA, and if not repaired, chromosomal replication will be altered in activated proliferating cells. The use of alkylating agents in the setting of transplantation is time dependent and is effective just before or during the activation of the immune system by antigen. Cyclophosphamide has been shown to have a greater effect on B-cells rather than T-cells, thereby inhibiting the humoral response to a greater degree.

Antibiotics

These agents (actinomycin D, mitomycin C, puramycin, chloramphenicol) inhibit either nucleic acid or protein synthesis.

Cyclosporin acts by inhibiting the production of IL-2, which results in an inhibition of the proliferation of T and B lymphocytes. Cyclosporin is widely prescribed for transplantation patients due to the clinical advantage of potent immunosuppression with limited myelosuppression.

FK-506 (Tacrolimus) is an agent that acts by inhibiting the production of IL-2 which prevents the proliferation of T and B lymphocytes.

Mycophenolate mofetil is rapidly converted to mycophenolic acid which selectively inhibits T and B cell proliferation. Mycophenolate mofetil has an advantage over azathioprine because it does not damage chromosomes.

Lymphocyte Depletion agents

Antilymphocytic globulin (ALG) is an agent that binds to circulating T-lymphocytes and the cells coated with the ALG are lysed and cleared by the reticuloendothelial system. ALG is more commonly used for renal transplantation, showing little to no benefit for liver or bone marrow transplantation.

Radiation

Total lymphoid irradiation or total body irradiation is based upon the immunosuppression observed after this procedure was used in patients with Hodgkin's lymphoma. The radiation causes breakdown in the nucleic acid structure, and the effect is time dependent since there are systems within all cells for the repair of DNA. Since the radiation affects those cells in M or G₂ phase, those cells in G₁ or S phase are resistant.

Monoclonal antibodies

A murine monoclonal antibody is available to deplete the circulating CD3 lymphocytes. This antibody reacts with the T3 recognition site of the T-lymphocytes and blocks the recognition of the Class I and II antigens. This leads to

prevention of the activation of the effector lymphocytes. This antibody has been useful in the treatment of rejection of renal, pancreatic, hepatic, cardiac, and pulmonary whole organ transplantations.

5 Steroids- such as the glucocorticoids are widely used in transplantation in combination with other drugs. As well as providing antiinflammatory therapy, corticosteroids suppress immune function by inhibiting the activation of T cells. Corticosteroids affect the inflammation within the airways by decreasing growth and development of mast cells, inducing apoptosis, suppressing lymphocyte generation of IL-5 and other cytokines, inhibiting some mediator release, inhibiting cytokine
10 production, inhibiting the transcription of cytokines (for example IL-8, TNF- α , prototypic antiviral chemokine (regulated-on-activation normal T-expressed and secreted, RANTES), and GM-CSF), and inhibiting nitric oxide synthesis. Steroids are highly effective in the early induction and maintenance regimens and are first
15 line therapy in acute allograft rejection.

Blood transfusions can be used to cause allosensitization if the recipient is exposed to donor antigens in the presence of azathioprine. In this way, induction of a specific degree of hyporeactivity against graft antigens can result by a potential suppressor cell phenomena.

20 Limitations of Immunosuppressive Therapies due to Lack of Efficacy

As suggested, the efficacy of immunosuppression is a balance between prevention of graft rejection or graft versus host disease and subjecting a patient unnecessarily to blunted immune defenses to ward off infections. All too often, this balance is not achieved and on one end the patient succumbs to infections or on the
25 other the graft is rejected. It has been estimated that 30% of the transplantation patients are in this category.

Limitations of Immunosuppressive Therapies due to Toxicities or Undesired Side Effects

30 Antiproliferative Agents

Azathioprine is associated with suppression of bone marrow production, and blood disorders including anemia, thrombocytopenia, and leukopenia.

Hepatotoxicity occurs in a dose-independent manner, and is irreversible.

Azathioprine is associated with chromosome damage and therefore is mutagenic.

35 Methotrexate and aminopterin are associated with bone marrow suppression, mucosal breakdown, gastrointestinal bleeding, megaloblastic hematopoiesis.

Alkylating Agents are associated with stomatitis, nausea, vomiting, diarrhea, skin rash, anemia, and alopecia. Specifically, cyclophosphamide has been associated with fluid retention, hemorrhagic cystitis, and cardiac toxicity.

Cyclosporin is associated with gingival hyperplasia, hirsutism, tremor, hypertension, hyperkalemia, hepatotoxicity, hyperglycemia, hypomagnesiumemia, hypercholesterolemia, hypertriglyceridemia, and hyperuricemia, nausea and gastrointestinal irregularities, and renal dysfunction. Nephrotoxicity associated with cyclosporin manifests as tubular necrosis, interstitial fibrosis, and tubular atrophy.

FK506 is associated with neurotoxicity, nephrotoxicity, and disturbances of glucose metabolism. The major neurotoxic symptoms are reversible and dose dependent and include headache, tremors, parasthesias, insomnia, increased sensitivity to light, mood changes, aphasia, and seizures. There has been a suggested association of FK-506 with cardiomyopathy and it is contraindicated in pregnancy.

Lymphocyte Depletion Agents

ALGs are associated with anemia, thrombocytopenia, and allergic reactions including urticaria, anaphylactoid reactions, serum sickness, joint pain, fever, and malaise.

Radiation is associated with higher incidence of infections and chromosomal breakage and mutations.

Monoclonal antibody therapy has been associated with the production of human anti-mouse antibodies (HAMA) in 80% of the treated patients and the sensitization rate is 15-40% thus limiting retreatment rates. Side effects are fever, chills, nausea, vomiting, headache, dyspnea, wheezing, pulmonary edema, tachycardia, hypotension, aseptic meningitis, seizures, and coma. These symptoms are related to the inordinate release of cytokines TNF, IL-1, and interferon-gamma. Although these symptoms can be reduced by pretreatment with steroids, acetaminophen, or diphenhydramine the HAMA response precludes repeated use.

Steroids- Glucocorticoid associated side effects include increased appetite, weight gain, fluid retention, acne, ecchymosis, development of cushoid facies, hypertension, hyperkalemia, diabetes, hyperglycemia, hyperosmolar state, hyperlipidemia, hepatic steatosis, atherosclerosis, myopathy, aseptic necrosis, osteoporosis, ulcers, pancreatitis, pseudotumor cerebri, psychosis, glaucoma, cataract formation, vascular necrosis, increased susceptibility to infection, impairment of the hypothalamus-pituitary axis, decreased thyroid hormone serum binding proteins, and impaired wound healing.

Complications of Immunosuppression

In addition to the above listed toxicities and undesirable side effects, potent immunosuppression as required in the transplantation setting leads to prolonged immune compromise and predisposes the patient to infections (80% of the patients) and cancer (ranging between 10-40% of the patients). This risk has been proposed to result from impaired immune surveillance mechanisms, chronic antigenic stimulation, reactivation of latent oncogenic viruses and the direct oncogenic effects of the immunosuppressive agents.

Moreover, 40% of the deaths of transplant patients are attributable to the complications of infections or a combination of infection and graft rejection. The infections experienced by transplant patients are 50% bacterial, 30% viral, 15% fungal. Some of the common bacterial infections are Staphylococcus aureus, Staphylococcus epidermidis, and gram-negative rods in line sepsis. Urinary tract infections, pneumonias, wound infections, and surgical infections (including cholecystitis, appendicitis, diverticular disease, ulcer, etc.). Common viral infections include cytomegalovirus, Epstein-Barr virus, Herpes Simplex. Virus, and varicella zoster virus. Further, common fungal or protozoan infections include Candida albicans, Aspergillus flavus, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Norcardia asteroides, and Pneumocystis carinii.

Description of Mechanism of Action Hypotheses for Future Immunosuppressive Drug Development

The majority of the hypotheses for future therapeutic interventions for graft rejection and graft immunoreactivity are based upon the understanding the immunologic mechanisms that cause and perpetuate the rejection within the graft.

A gene, genes, or gene pathway involved in the etiology of transplantation or immunosuppression or associated disorders or potential sites for targeted drug therapy of transplantation are depicted in Table 9 with the specific gene list in Table 4. Current candidate therapeutic interventions in development for the treatment of anemia are listed in Tables 42 and 43.

E. Pain Associated with Inflammation

Description of Pain Associated with Inflammation

Pain associated with inflammation can be caused by pathologic processes in somatic structures or viscera, or by prolonged dysfunction of parts the peripheral nervous system.. Pain associated with inflammation may be the result of recurrent injuries, trauma, headache, arthritis, chronic obstructive pulmonary disease, psoriasis, or other pathologies. Pain associated with inflammation may be acute or chronic depending on the level and extent of the inflammation.

Current therapies for Pain Associated with Inflammation

Therapeutic management of pain resulting from inflammation includes a three step ladder approach: non-opioid analgesics are stepwise prescribed in combination with moderate to potent opiates. The guidelines call for a determination by the patient and the physician of pain relief. Broadly speaking, the guidelines are as follows: mild pain is treated with non-opioid analgesics, moderate or persisting pain is treated with a weak opioid plus non-opioid analgesics, and severe pain that persists or increases is treated with a potent opioid plus non-opioid analgesics.

Analgesics: Typically, pain associated with inflammation can be controlled with NSAIDs including but not excluded to, salicylates, para-aminophenol derivatives, indole and indene derivatives, heteroaryl acetic acids, arylpropionic acids, anthranilic acids, enolic acids, or alkanones. Antiinflammatory agents such as cyclooxygenase inhibitors, lipoxygenase inhibitors, and others can be used to block the inflammation physiological pathway which mediate pain and the progression of the disease. However, because these drugs are limited in their efficacy in advanced or more severe stages of arthritis, these agents are add-on therapies.

NSAIDs derive their principle mechanism of action by the inhibition of prostaglandin and leukotriene synthesis. These compounds inhibit key enzymes in the biosynthetic pathway, i.e. cyclooxygenase. There are drugs that selectively inhibit isoforms of cyclooxygenase 1 and 2 (COX-1, COX-2) which enhances patient tolerance due to the prevalence of COX-2 induction occurs in inflammation mediated by cytokines and others.

Further, pyrimidine synthesis inhibitors can be used as an antiinflammatory agent in arthritis, e.g. leflunomide.

Limitations of Current Therapies for Pain Associated with Inflammation

Limitation of Therapies for Pain Associated with Inflammation due to Low efficacy

The therapies discussed above are limited to the slowing or retarding the progression of arthritis. As degeneration of the joints progresses, and irreversible damage occurs, the options become limited. Thus, therapies for arthritis are aimed at reduction of manifestation of symptoms by controlling the clinical manifestations of inflammation.

Limitations of Therapies of Pain Associated with Inflammation due too Toxicity or Undesired side effects

Analgesics associated side effects include dyspepsia, gastric or small bowel bleeding, ulceration, renal insufficiency, confusion, rash, headache, hepatic toxicity. NSAIDs also reversibly inhibit platelet aggregation and prolong bleeding time.

5 Description of Mechanism of Action Hypotheses for Future Pain Associated with Inflammation Drug Development

 The persistence of pain most likely involves a cascade of pathological neurochemical events that lead to abnormal sensory hyperexcitability and excitotoxicity. The genes listed in Figure 1 are part of a pathway are all involved in
10 producing prostaglandins or leukotrienes, which are two potent mediators of inflammation. Inordinate levels of prostaglandins have been implicated in pain associated with inflammation, and several drugs target this branch of the pathway, to inhibit the action of leukotrienes. When a cell receives a pro-inflammatory stimulus, such as tumor necrosis factor, membrane phospholipids, or interleukin-1, as shown in
15 the figure, membrane phospholipases are activated, and arachidonic acid is released from membrane phospholipids into the cell. The liberated arachidonic acid is then metabolized either by the cyclooxygenase enzymes, which leads to the production of prostaglandins, or the lipoxygenase family of enzymes, which leads to the production of leukotrienes. There are several types of prostaglandins and leukotrienes, and
20 many of the enzymes listed here function to convert one form into another.

 The presence of leukotrienes and prostaglandins can lead to a persistence of neural hyperexcitability involving a sequence of neuroplastic events.

 A gene, genes, or gene pathway involved in the etiology of pain or associated disorders or potential sites for targeted drug therapy of pain are depicted in Table 9
25 with the specific gene list in Table 4. Current candidate therapeutic interventions in development for the treatment of pain associated with inflammation are listed in Table 44.

30 F. Psoriasis
 Description of Psoriasis

 Papulosquamous skin disorders have diverse etiologies and include psoriasis, Reiter's syndrome, pityriasis rosea, lichen planus, pityriasis rubra pilaris, secondary syphilis, mycosis fungoides, and ichthyosiform eruptions.

 Psoriasis is a genetically determined, chronic epidermal proliferative disease
35 with an unpredictable course. Psoriasis appears as erythematous plaques with silvery, mica-like scales, and is usually nonpruritic. The plaques appear anywhere on the body and almost never involves the mucous membranes. There are variations of psoriasis including guttate psoriasis, inverse psoriasis, pustular psoriasis,

erythroderma, and psoriatic arthritis. There is an increased prevalence of psoriasis in subjects with the HLA antigens BW17, B13, and BW37. Further, 30% of cases have a family history of psoriasis. The Koebner phenomena is a hallmark characteristic of psoriasis, e.g. intense trauma (scratches or surgical incisions) to the skin induces new linear papulosquamous lesions.

This multifactorial disease is characterized by an accelerated cell cycle in an increased number of dividing cells that results in rapid epidermal cell proliferation. It is estimated that 4-5 million Americans have psoriasis, 100,000 have severe cases, and 1 in 20 have psoriatic arthritis.

Current Therapies for Psoriasis

The goals of the therapeutic regimens is to limit the epidermal proliferation underlying the dermal inflammation. There are both topical and systemic treatments available, however in either category the treatment suppresses the condition for only as long as is administered. The treatment of psoriasis entails a stepwise increase of extent of the therapy ranging from topical applications to phototherapy to systemic interventions to prevent the epidermal proliferation.

In the first step topical treatments include corticosteroid ointments, vitamin D containing ointments, preparations containing coal tar or anthralin, salicylic acid containing ointments, and various other moisturizers and bath solutions. These steps are aimed at reducing the itching, scaling, and progression of the lesions.

In the second step, phototherapy other than natural sunlight can be used to thwart the epidermal cell proliferation. In these cases, ultraviolet light is administered to affected areas or uniformly to the body. In phototherapy, light delivered to the skin activates porphyrin molecules. These activated molecules transfer their energy to form cytotoxic singlet oxygen leading to lethal alteration of cellular membranes and subsequent tissue destruction. In UVB therapy, UVB light is administered alone or with ointments containing coal tar, anthralin, or salicylic acid. UVA light is administered with psoralen.

In the third step of therapeutic regimens for psoriasis, systemic agents are administered to those cases refractory to the previously described first two steps. These compounds include retinoids, methotrexate, hydroxyurea, cyclosporin, azathioprine, 5-fluorouracil, cyclophosphamide, vinblastine, dapsone, and sulfasalazine.

Limitations of Current Therapies for Psoriasis

The main limitation of the current therapies for psoriasis is that the drugs are only efficacious during the administration. Further, periods of remission and

outbreaks are difficult to impossible to predict. It has been shown that patients must rotate their treatments to retain efficacy. This can lead to missed schedules and requires patient education. Lastly, for all the listed therapies there is unreliable efficacy in their ability to stop proliferation and inflammation of the lesions.

5 Toxicities of the current therapies include the following: phototherapy can lead to other skin lesions and sunburn. Cytotoxic agents used as immunosuppressive agents including methotrexate, 5-fluorouracil, cyclophosphamide, and vinblastine have associated side effects including hepatic compromise including hepatic fibrosis, ascites, esophageal varices, cirrhosis, pneumonitis, myelosuppression, 10 (cyclosporine: renal insufficiency anemia, hypertension).

A gene, genes, or gene pathway involved in the etiology of psoriasis or associated disorders or potential sites for targeted drug therapy of psoriasis are depicted in Table 9 with the specific gene list in Table 4. Current candidate therapeutic interventions in development for the treatment of psoriasis are listed in 15 Table 45.

G. Atherosclerosis

Description and Potential Intervention of Atherosclerosis

Atherosclerosis is a complex combination of hyperlipidemia, injury to the 20 endothelium, and inflammation. The interaction of these multiple processes in association with local genetic and hemodynamic influences may promote the formation of atheromatous plaques as a reparative response of the arterial wall. Atherosclerotic plaques are composed of thrombogenic lipid-rich core protected by a fibrous cap comprising smooth muscle cells and inflammatory cells. The 25 inflammatory cells are predominantly macrophages. As atherosclerotic plaques build blood flow is reduced creating ischemia in tissues down stream from the area of the plaque.

In another model, the stenosis created by the plaques may be a part of the resulting ischemic event. Frequently, less obstructive but more vulnerable plaques 30 occur which are characterized by a thinned fibrous cap, marked lipid accumulation, a large number of macrophages, and a smaller amount of smooth muscle cells. It has been proposed that since these plaques are more prone to rupture creating contact with the highly thrombogenic materials of the lipid-rich nucleus of these lesions, thrombosis is stimulated.

35 Advanced atherosclerotic lesions are caused by a series of cellular and molecular events involving replication of smooth muscle cells and macrophages on the vessel wall. The interaction of these cells with the T lymphocytes can lead to a fibroproliferative response. Large amounts of connective tissue produced by these

smooth muscle cells consist of macrophages, T lymphocytes, smooth muscle cells, connective tissue, necrotic residues, and varying amounts of lipids and lipoproteins.

Endothelial cells maintain the vessel surface in a non-thrombogenic state, preventing platelet and leukocyte adhesion, and act in maintaining the vascular tonus by releasing nitric oxide, prostaglandin, and endothelin. These cells also produce growth factors, cytokines, and chemokines to maintain the integrity of the collagen- and proteoglycan-rich basement membrane. Changes in some of these functions may trigger cell interactions with monocytes, platelets, smooth muscle cells, and lymphocytes. Hyperlipidemia and hypercholesterolemia are sufficient to induce dysfunction of the endothelial modulation of the vasoactive reactions and arteriolar tonus.

The inflammatory mechanisms involved in the initial events or atherosclerosis are classic components of a specialized type of chronic inflammatory response that precedes the migration and proliferation of smooth muscle cells of the vessel wall. The formation and accumulation of foam cells in the intima leads to the first stage of the atherosclerotic lesion. In this stage, the accumulation of fatty striae consisting of a mixture of macrophages, lipids, and T lymphocytes representing a purely inflammatory response. If the stimulating agent is maintained, i.e., hyperlipidemia, hypercholesterolemia, or other risk factor, then the protective inflammatory response will also persist and may become deleterious to the cells lining the arterial wall. This condition may lead to an intermediate lesion that may contain multiple smooth muscle cell layers, macrophages, and T lymphocytes. A fibrous capsule is formed covering the contents of the lesion.

There is evidence to suggest that the inflammatory process and specific immune mechanisms are involved in atherogenesis. At sites close to the plaque rupture, inflammatory processes are observed resulting from T cell-dependent autoimmune response. This may lead to inflammatory reactions participating in the destabilization of the fibrous cap. Immunoglobulins, T lymphocytes, and macrophages are found in the plaques. B lymphocytes and plasmocytes may also be detected in the adventitia adjacent to the plaques. Autoimmune reactions against the oxidized lipoproteins have been observed. The macrophages are transformed into foam cells and in the presence of LDL, form immunocomplexes with the LDL by Fc fragments of the immunoglobulins. These LDL immunocomplexes can induce numerous metabolic and functional changes which can directly or indirectly damage the endothelial cells leading to the progression of the atherosclerotic lesion.

Despite the evidence of the involvement of the immune system in atherogenesis, the complexity of the immune reactions and response impairs the clarification of the involvement of these mechanisms at the various stages of

atherosclerosis. The sequence of immune response event suggests an initial mechanism to respond to injury. However, this protective inflammatory response in the presence of persistent stimulus and the formation of a fibroproliferative response can be amplified.

5 Attempts to modify the specific cell interactions with growth factor mediators or intracellular signalling molecules has provided a window to the potential prevention or regression of the lesions.

A gene, genes, or gene pathway involved in the etiology of atherosclerosis or associated disorders or potential sites for targeted drug therapy of atherosclerosis are depicted in Table 9 with the specific gene list in Table 4. Current candidate
10 therapeutic interventions in development for the treatment of atherosclerosis are listed in Table 46.

Endocrine and Metabolic Disease

15 Included in the description below are endocrinologic and/or metabolic diseases, disorders, or syndromes. They include diabetes, diabetes insipidus, obesity, contraception (not a disease but a common reason for taking steroid drugs), infertility, hormonal insufficiency related to aging, osteoporosis, acne, alopecia, adrenal dysfunction, thyroid dysfunction, and parathyroid dysfunction. Application
20 of the methods of this invention to these diseases is described.

A. Diabetes Mellitus

Carbohydrate metabolism in mammals is controlled by a unique interplay of hormones, neurotransmitters, and other physiological influences to ensure a constant
25 supply of metabolic fuel is available to the tissues. The two main hormones that regulate carbohydrate balance are insulin and glucagon. Both hormones are produced in the pancreas; β -cells produce insulin, α -cells produce glucagon. Insulin in the fuel excess state stimulates storage of the available metabolic precursors into glycogen and lipids; glucagon in the fuel deficient state stimulates the movement of
30 the fuel stores to available metabolic precursors. When regulation of insulin or glucagon is abnormal there are pathologic changes.

Type II Diabetes (Diabetes Mellitus; DM) is a heterogeneous disorder of carbohydrate metabolism characterized by absolute or relative insulin deficiency alone or in combination with insulin resistance (sensitivity). DM is associated with
35 hyperglycemia and consequent polyuria and polydipsia.

There are two forms of the disease, insulin-dependent diabetes mellitus (IDDM) which accounts for approximately 10% of the DM cases in the United States, and non-insulin-dependent diabetes mellitus (NIDDM) which accounts for

the remaining diagnosed cases. The incidence rate for all cases of DM in the U.S. is approximately 440 per 100,000. Type I (juvenile onset) diabetics produce little or no insulin and may be severely hyperglycemic if untreated. They are entirely dependent on exogenous insulin administration. NIDDM (maturity or adult onset, nonketotic DM) patients retain significant capacity to secrete insulin, do not exhibit ketosis, and are not dependent on exogenous insulin for immediate survival. Within the pancreas, the β -islets cells are lost, stop producing or secreting insulin in patients with IDDM, but remain functional in patients with early stage NIDDM. In both cases of DM, glucagon opposes the effect of insulin on the liver by stimulating glycogenolysis and gluconeogenesis, but glucagon has little if no effect on the peripheral utilization of glucose. In the diabetic patient with insulin deficiency or insulin resistance and hyperglucagonemia, there is an increase in hepatic glucose production, a decrease of peripheral glucose uptake, and a decrease in the conversion of glucose to glycogen in the liver.

Broadly, the physiologic changes stimulated by insulin, the primary hormone responsible for specific uptake of glucose from the periphery to tissues, is to increase the available storage of glucose into glycogen stores. In the liver, insulin stimulates the uptake and storage of glucose as glycogen, and inhibits hepatic gluconeogenesis and glycogenolysis. In skeletal muscle, insulin stimulates glucose uptake and storage as glycogen and amino acids in protein and inhibits release of gluconeogenic precursors (e.g., alanine, lactate and pyruvate) to the hepatic circulation. In adipose tissue, insulin stimulates the glucose uptake and metabolism to glycerol (the backbone of triglycerides for storage in fat droplets) and inhibits the flow of gluconeogenic precursors to the hepatic circulation, e.g. glycerol and nonesterified fatty acids. Insulin inhibits the breakdown of triglycerides, glycogen, protein and the conversion of amino acids to glucose (gluconeogenesis).

In the intracellular process of storage of glucose as glycogen in the liver, insulin stimulates the glycogen synthase complex and inhibits glycogenolysis. However, in the insulin deficient or insulin resistant patient, glycogen stores are depleted and replaced with stores of ketone bodies (see below).

In the intracellular process of storage of amino acids in muscle as protein, insulin stimulates the production of amino acids and their incorporation into protein. In the absence of insulin, the amino acids stored in the muscle or other tissues, protein manufacture is reduced, and all available amino acids are metabolized to pyruvate, oxaloacetate, and β -ketoglutarate. The pyruvate can be converted to acetyl-CoA which can be further metabolized to acetoacetate, free fatty acid-CoA, or enter the cholesterol synthetic pathway via HMG CoA. In this case, there is production of ketones, fatty acids, and cholesterol.

In the intracellular process of storage of metabolic fuel within the adipose tissue, insulin stimulates lipoprotein lipase. Lipoprotein lipase is synthesized primarily in fat and muscle, and when secreted into the extracellular space, the enzyme is associated with the surface of endothelial cells. Lipoprotein lipase hydrolyzes free fatty acids from triglyceride-rich lipoproteins (i.e. chylomicrons, very low density lipoproteins). Free fatty acids liberated from the lipoproteins are then taken up by adipose tissue, esterified into triglycerides for storage in fat droplets or adipocytes. Insulin stimulates the synthesis and secretion of lipoprotein lipase, inhibits lipolysis of triglycerides stored in adipose tissue, and promotes glucose uptake into the fat stores to provide a glycerol substrate within the adipocytes for esterification of the fatty acids.

In cases whereby there is limited insulin supply or responsiveness, there is an enhanced production of free fatty acids. The excess of free fatty acids stimulates the production of ketones (β -hydroxybutyrate, acetoacetate) and the release of ATP.

Diabetic ketoacidosis (DKA) describes a clinical situation whereby there is a severe elevation of ketones in the tissues and peripheral circulation with concomitant hyperglycemia. In hepatocytes, mitochondria produce ketone bodies, which form as the result of β -oxidation of fatty acids. Glucagon further stimulates the hepatic ketogenic state; glucagon lowers malonyl coenzyme A levels (the first enzymatic step in the production of fatty acids) which in turn stimulates the activity of carnitine acyltransferase I, an enzyme that translocates fatty acids from cytosolic to intramitochondrial spaces. The fatty acids once in the mitochondria are converted in the absence of glucose to ketones.

The production of excess ketones in DKA is uncontrolled: normally insulin stimulates the ketoacid tissue uptake and the high concentration of ketones themselves saturates tissue uptake. However, in DKA, the only resultant mechanism to remove or excrete excess ketones is via the kidneys. Hyperketonuria causes osmotic diuresis, which in turn causes intravascular volume depletion and dehydration, leading to urinary electrolyte loss. The hyperosmolarity exaggerates the intracellular dehydration.

The hallmark of NIDDM is peripheral tissue insulin resistance. The characteristic post-insulin receptor defect has been difficult to target therapeutically, however, there are working hypotheses to be exploited during drug development. One theory to explain how insulin resistance comes about is the single gateway theory. In the liver, it is thought that insulin is acting not directly on the hepatocytes, but through an indirect means. In this theory, insulin resistant fat cells over produce free fatty acids. It is the free fatty acids that circulate to the liver,

muscle, and others tissues to mediate insulin resistance by a yet unknown mechanism of action.

Another explanation of insulin resistance is free fatty acid response within adipose tissue. In this theory, free fatty acids stimulate the adipocyte production of TNF α and TNF α creates insulin resistance locally and distally within other peripheral tissues. It is thought that TNF α mediates insulin resistance within adipose tissues by stimulating de-differentiation by inhibiting peroxisome proliferator receptor- γ (PPR- γ) and CAAT-enhancer binding protein α (CEBP α) while activating serine-threonine phosphorylation via the MAP kinase cascade. TNF α has been shown to stimulate lipolysis. Further, TNF α stimulates apoptotic signals by activating capases. Within the skeletal muscle TNF α inhibits insulin stimulated glucose uptake, and directly affects the insulin signaling pathway; it stimulates phosphorylation of the IRS-1; and inhibits PPR- γ and CEBP α . An example of the importance of TNF α on the mediation of insulin resistance are recent studies in adipocyte macrophages whereby it has been shown that TNF α has a direct effect on macrophages metabolism (a shift from glucose utilization to free fatty acid production) and a direct effect on PPR- γ and CEBP α .

Type II DM is associated with metabolic syndrome X, also referred to as insulin resistance syndrome, or metabolic syndrome. This syndrome is characterized by hypertriglyceridemia, low serum high density lipoprotein (HDL) and cholesterol, hypertension, central obesity, defective fibrinolysis, and arteriosclerosis. Syndrome X, "the deadly quartet" of obesity, NIDDM, hypertension, and dyslipidemia are common metabolic disorders that have been shown to predispose the patient to early cardiovascular disease, including but not limited to coronary artery disease, heart failure, or congestive heart failure. In these cases, the pancreatic β -cells produce insulin, but the peripheral tissues are physiologically unresponsive to insulin. Thus, the mechanisms of insulin deficiency are active and the resultant hyperglycemia, hyperlipidemia, and others described are present. Clinically, the patient exhibits the signs and symptoms of NIDDM, and unfortunately few therapeutic alternatives are available. Table 50 lists the current candidate therapeutic interventions that are in development for the treatment of IDDM and NIDDM.

Metabolic Syndrome X- It is well known that individuals who are diagnosed with metabolic syndrome X progress to a diagnosis of IDDM. One explanation of the transition of insulin independent to insulin dependent DM is that the overactive, uncontrolled pancreatic β -cells in NIDDM may generate oxygen free radicals that are deleterious to the β -cells and they undergo apoptosis. Another theory that may explain the loss of β -cells is that free fatty acids produced in adipose, hepatic, and

other tissues may compromise the activity of the functioning pancreatic β -cells and ultimately leads to β -cell apoptosis and death. Lastly, the overexpression of TNF α within adipose tissue may activate apoptotic signals within the pancreatic β -cells.

Therefore, in cases of NIDDM, it is clinically advantageous to blunt the progression of the disease to syndrome X. Therapeutic alternatives to treat NIDDM are as follows: 1) diet modifications that are aimed at lowering the daily intake of glucose (carbohydrates) and lipids; 2) low doses of exogenous insulin can be used to inhibit the patient's production and secretion of insulin from the pancreatic β -cells; 3) oral hypoglycemic agents, e.g. sulfonylureas (first and second generations), biguanides, thiazolidinediones, and α -glucosidase inhibitors. Once in syndrome X, there are many other therapeutic alternatives that are added to the regimen to treat the "deadly quartet" as described above.

Novel therapeutic alternatives are required to be developed to meet the need of the population of NIDDM as well as those individuals in which progression to syndrome X has occurred. Table 56 lists the current candidate therapeutic interventions in development for the treatment of one or more of the deadly quartet that is part of metabolic syndrome X.

Many human primary and metastatic tumors express critical proteins required for the maintenance of growth and dedifferentiation along with proteins that may inhibit growth or enhance terminal differentiation. For example, breast adenocarcinomas express at significant levels peroxisome proliferator activated receptor gamma (PPAR γ), that when activated by a specific ligand, will induce terminal differentiation of malignant breast epithelial cells. Although, specific activators of PPAR γ have been developed for the treatment of NIDDM, the antiproliferative and terminal differentiation effect may be exploited for the development of anti-neoplastic agents. Further, agents affecting the PPAR γ pathway may be desirable candidate therapeutic interventions for cancer and DM. Current candidate therapeutic interventions for the treatment of cancer are listed in Table 24.

Besides metabolic syndrome X, there are other chronic late complications of IDDM and NIDDM including retinopathy (proliferative and nonproliferative), nephropathy, neuropathy (including symmetric distal polyneuropathy, asymmetric neuropathy, cranial mononeuropathy and mononeuropathy multiplex), peripheral mononeuropathy and, neuromuscular syndromes and autonomic neuropathy, cardiovascular disease, and skin ulcers due to vascular disease. In cases with loss of sensation in the extremities, there is a predisposition to repeated and undetected trauma. Diabetics are also at increased risk for cardiovascular disease. These complications are only partially reduced by achieving tight control of blood glucose levels.

B. Diabetes Insipidus

The polyuric syndrome in which there is a dysfunction in the antidiuretic hormone (ADH, often referred to as vasopressin (AVP)) signalling pathway, with an
5 loss of ADH activity, and is termed diabetes insipidus (DI). Since ADH is responsible for the appropriate concentration and water conservation in the body, clinical manifestations of this disorder include: polyuria, near-continuous thirst, nocturia, hypertonic encephalopathy, circulatory collapse, and hypernatremia. These symptoms can lead to life-threatening syndromes.

10 In DI, there is a vasopressinergic deficiency or the target organs are unresponsive to ADH (nephrogenic diabetes insipidus). The etiology of the disorder includes disease processes of the supraoptic nuclei, paraventricular nuclei, the hypothalamohypophysial tract, or the pituitary gland. Although 30% of the cases
15 are attributed to neoplastic lesions of the hypothalamus, 30% are post-traumatic, and 30% are idiopathic, with the remaining 10% being attributed to vascular lesions, infections, systemic diseases such as sarcoidosis that affect the hypothalamic function, and mutations in the ADH gene preprohormone processing pathway.

Treatment of DI depends on the level and extent of the vasopressinergic deficiency. In cases, restoration of fluid balance and control of dehydration is
20 paramount. In some cases of partial loss of ADH, relief of symptoms can be attained through the use of vasopressinergic agonists, candidate therapeutic interventions that enhance vasopressin secretion (e.g. clofibrate), or agents that increase the renal response to vasopressin (e.g. chlorpropamide).

In cases of nephrogenic DI, there is an inability of the renal cells to respond
25 to vasopressin. In one form of this condition, there is congenital defects of the vasopressinergic receptor V2, preventing the ADH stimulation of adenylate cyclase and is an X-linked autosomal dominant genetic condition. In another form of nephrogenic DI, there are mutations in the autosomal gene for aquaporin-2 which produce a nonfunctional versions of this water channel.

30 Although DI is more common, hypersecretion or over-activity of the ADH pathway leads to a syndrome termed inappropriate hypersecretion of ADH (SIADH). In this syndrome there is profound hyponatremia. This syndrome can occur in patients with cerebral disease (cerebral salt wasting) or pulmonary disease (pulmonary salt wasting), in some cases whereby a tumor is hypersecreting
35 vasopressin, or in the absence of complicating disease. In these cases, patients with inappropriate hypersecretion or vasopressin can be successfully treated with agents or candidate therapeutic interventions that interrupt the vasopressinergic signal, for example, meclocycline, an antibiotic that reduces the renal response to vasopressin.

C. Obesity

According to a commonly accepted definition, obesity refers to a condition by which more than 20% or 25% of body weight is due to fat in men and women, respectively. Another, more reliable, index of fat distribution is the body mass index (BMI) which is calculated as the body weight divided by the square of the height (normal range being 20-25 kg/m²). Obesity is a serious illness that can lead to many complications including hypertension, diabetes, cancer, degenerative arthritis, elevated cholesterol, gallstones or inhibited bile secretion, heart attacks and other cardiovascular disease, strokes, sleep disorders, and psychiatric illnesses including anxiety and depression. There is a strong genetic component to obesity, as well as strong correlations between obesity and socioeconomic status.

Tables 5 and 10 lists the possible genes and gene pathways involved in the manifestation of obesity. Specifically, there are two gene pathways that may be associated with a genetic predisposition to obesity, they are leptin and its receptor, and peroxisome-proliferator-activated receptor γ 2 (PPAR γ 2). In the first, the lipostatic hypothesis of obesity achieved prominence for a potential mechanism of inordinate eating. It was determined in mice lacking a specific gene, the *ob* gene, did not become satiated after eating and ultimately became obese and diabetic. The product of this gene is a 167 amino acid protein called leptin. Leptin acts as a hormone to reduce food intake and increase energy consumption. The leptin receptor is encoded by the *db* gene. Mice lacking the *db* gene are also obese, but have high levels of circulating leptin. The leptin receptor is found in two forms, the short and long form which are the result of alternative splicing. The long form is found in the hypothalamus.

The mechanism of leptin and leptin receptor dysfunction creating obesity is thought to occur by (i) interfering with the transport of leptin into the ENDOCRINE AND METABOLIC, (ii) impairing leptin receptor signal transduction, (iii) impairing downstream mediators of leptin action, or (iv) causing obesity by a leptin-independent mechanism – for example a mechanism that originates downstream of leptin or that bypasses leptin. Each of these hypotheses invokes a set of candidate genes (with considerable overlap) and implies up or down variation in allele function.

The genes with potential affect on leptin and leptin associated activity are leptin receptor (OB-R), melanocortin 4-receptor (MC4-R), pro-opiomelanocortin (POMC; the precursor of α -melanocyte stimulating hormone), and prohormone convertase 1 (PC1). Two lines of evidence suggest that variation in these genes may affect leptin resistance. First, each gene has been strongly implicated in the leptin

signaling pathway by *in vitro* data. Specifically, PC1 participates in the processing of the prohormone POMC to the α -melanocyte stimulating hormone (α -MSH), which signals decreased food intake in response to leptin. This signal is transmitted through MC4-R, the receptor for α -MSH. Second, mutations in each of these genes have been associated with obesity in humans and, except PC1, in rodents as well. Leptin signaling could be affected by polymorphisms that affect protein levels or function. Furthermore, there may be polymorphisms in the promoters of all four genes as well as the genomic locus of the leptin receptor and three genes implicated in the signal transduction pathway immediately downstream of the leptin receptor.

Other genes involved in the leptin signal include Neuropeptide Y. Each gene in this set has the potential to modulate the biological function of leptin. Neuropeptide Y, which stimulates food intake through the Y1 and Y5 receptors (and possibly others), is inhibited by leptin. Agouti-related protein inhibits MC4-R signaling and is also down-regulated by leptin. Like NPY, the melanin-concentrating hormone has been shown to stimulate feeding. These genes differ from those above in that mutations have not been associated with obesity in humans (although mutations in the neuropeptide Y1 receptor and the agouti-related protein have been associated with obesity in rodents). With the exception of neuropeptide Y (NPY), where the coding region (but not genomic or promoter sequence) has been screened for polymorphism, these genes have not been studied extensively for variation in humans.

In the second gene pathway associated with obesity, PPAR- γ 2, is a transcription factor (described above and in Example 1) and has been demonstrated to be a key regulator of adipocyte differentiation and energy storage. PPAR- γ 2 is involved in the direction of differentiation of preadipocytes to adipocytes. In *in vitro* studies, over expression of PPAR- γ 2 leads the fibroblast cells to differentiate to adipocytes. Furthermore, phosphorylation of PPAR- γ 2 at a serine residue at position 114 reduces differentiation process mediated by PPAR- γ 2. This serine is contained within a mitogen activated protein kinase or related kinase, indicating an intracellular mechanism for the regulated control of adipocyte differentiation. In a recent study, it was determined that 4 of 121 obese subjects were identified as harboring a substitution of proline to a glutamine at amino acid position 115 as compared to none of the normal subjects having the substitution (Ristow et al, NEJM 339(14):953-959). Since the amino acid at position 115 is near to the serine phosphorylation site at 114, it is suggestive that such a substitution can be predisposing to aberrant PPAR- γ 2 activity.

Other genes that be involved in the genetic differences in obese versus normal weight subjects include signaling genes based on two observations. First,

although no human or rodent models are available to assess the affect of mutation on body mass, it has been shown that JAK2 and STAT3 knockouts are embryonic lethals. This would seem to indicate functions beyond regulation of body mass. Second, there is considerable redundancy in most signal transduction pathways, and there may be compensatory mechanisms to overcome any effects of polymorphism in JAK2 or STAT.

As depicted in Table 51, there are many new candidate therapeutic interventions in development. The targets include galanin, β 3-adrenergic receptor, neuropeptide Y, corticotropin releasing factor, and the cholecystokinin receptors.

D. Contraception

The most widely used oral contraceptives are estrogens and progestins alone or in combination. These agents are taken by women each day to prevent ovulation. The combination therapies are either mono-, bi-, or triphasic which are named as such to indicate the level of estrogen in each of the tablets, i.e. monophasic has the same amount, biphasic has two different doses, and triphasic has three. Progestins are delivered in the same tablet, and the ratio of estrogen to progestin allows for a reduction in the overall amount of steroids delivered to the subject as well as more closely approximates the natural steroid ratio during a menstrual period. The phase delivery of steroids to women wishing to block ovulation has limited the untoward side-effects progestins have on the cardiovascular system.

Unfortunately, although very effective, oral contraceptives are associated with undesirable side effects and toxicity. These effects falls into three categories: cardiovascular effects, cancer, and metabolic and endocrinologic effects.

Cardiovascular effects seen in response to oral contraceptives include estrogen increasing serum HDL while lowering serum LDL and progestins decreasing HDL and increasing LDL. This inordinate and unregulated change in the liporpotien balance in women can lead to hypertension.

Estrogen is a growth promoting hormone, and the estrogen found in almost all of the oral contraceptives has been studied for effects on or risk of ovarian, cervical, endometiral, and breast cancer as well as hepatocellular adenoma in women. However, studies have not conclusively demonstrated an association of higher rates of these types of cancers in women that have used oral contraception.

The metabolic and endocrine effects of oral contraceptives are increased fasting glucose levels, peripheral insulin resistance, higher incidence of gall bladder disease, and estrogen mediated increases of hepatic synthesis of serum proteins.

There are other side effects and disease risk that are associated with oral contraceptives that include increased risk of thromboembolism, nausea, vomiting,

dizziness, headaches, decreased libido, visual disturbances, depression, and post-pill amenorrhea. However, there are beneficial effects of oral contraceptives that include reduction of pelvic inflammatory disease, lower incidence of iron deficient anemia, symptomatic relief of endometriosis, improvement of acne and
5 dysmenorrhea, as well as decreased risk to develop ectopic pregnancies, uterine fibroids, and ovarian cysts.

Oral steroid contraceptives also interact with several other drugs and such interactions can lead to loss of efficacy and include altered drug absorption or metabolism. Any agent or compound that induces hepatic microsomal enzymes or
10 reduces the absorption can alter the effectiveness of the oral contraceptives and these include certain antibiotics, anticonvulsants, or antacids. Furthermore, agents that oppose the therapeutic effects of the oral contraceptives include anticoagulants, antidiabetics, and certain antihypertensives (guanethidine, and α -methyldopa).

There are other genes that one may correlate to candidate therapeutic
15 responses or safety and these include: blockade of implantation, blockade of sperm penetration into the egg, or blockade of sperm production.

As depicted in Table 52, there are many candidate therapeutic interventions that are currently in development to be of therapeutic benefit in contraception.

20 E. Infertility

Infertility is the involuntary inability to conceive a child. Infertility is the result of one or more of the following functions for the male or female including 1) adequate production of normal motile sperm, 2) ejaculation of sperm through a patent ductal system, 3) the sperm must be able to traverse an unobstructed female
25 reproductive tract, 4) the female must ovulate and release the ovum, 5) the sperm must be able to enter the ovum, 6) the fertilized ovum must be capable of developing and implanting in the appropriately prepared endometrium. Nearly 40% of the infertility cases, the male has a dysfunction or inadequate function.

Couples experiencing infertility have alternatives to alter their reproductive
30 capacity. Although many of the methods are mechanical and require a procedure, such as *in vitro* fertilization and sperm collection and concentration, there are agents that help a female to ovulate, such as antiestrogens and gonadotropins.

F. Hormonal insufficiency related to aging

35 As individuals age, their androgen and estrogen levels decrease. In some cases, estrogen and androgen replacement therapy has been useful to replenish the deficiency and restore the steroid hormone stasis. In these cases, the deficiency may be the result of a loss of the receptor affinity for the ligand, loss of the receptor

levels, reduction in the production of the steroids, or increased metabolic rates of these steroids. As the aging process continues, there may be a natural reduction in the function within the estrogen or androgen target tissues.

5 *G. Osteoporosis*

The condition in which there is bone matrix and mineral loss is termed osteoporosis. The loss of both of these components in bone results in the reduction of strength, and increased incidence of fractures and is characterized by a net excess loss of bone resorption over bone formation. Although there are multiple causes, the
10 most common is involutional osteoporosis which is associated with advancing age and menopause. Osteoporosis can also occur as a result of long periods of immobilization, space flight, parathyroid hormone and vitamin D deficiency, as well as in patients with excess glucocorticoids (Cushing's syndrome, or administration of glucocorticoids for the therapy of autoimmune disease, transplantation,
15 inflammatory diseases, arthritis, asthma, Crohn's disease, atherosclerosis, or infections with potent inflammatory responses such as hepatitis).

In osteoporosis accelerated normal bone loss can be reversed by estrogens. Estrogens inhibit the secretion of IL-1, IL-6, and TNF α . These cytokines enhance the production of osteoclasts, and in addition, estrogen inhibits the production of
20 TGF- β which is thought to mediate the apoptotic signal within osteoclasts. Although estrogen can reverse bone loss in patients with osteoporosis, the doses of estrogen required are associated with higher risk of myocardial infarctions, stroke, breast and endometrial cancers. However, as described above (under *Contraception*), estrogen in lower doses and given with progestins can be of
25 therapeutic benefit for osteoporosis and have a reduced toxicity profile.

Table 53 lists the current candidate therapeutic interventions that are in development for osteoporosis.

H. Acne

30 The most common form of noninfectious pustular skin disease is acne. It is an inflammatory skin condition affecting the pilosebaceous units and therefore is predominantly found on the face and upper trunk. Several factors can play a role in the progression of acne including 1) androgenic stimulation of the sebaceous glands, and 2) abnormal keratinization and impaction in the pilosebaceous
35 canals causing obstruction of the sebum flow, and 3) proliferation of anaerobic bacteria. Aggravating factors such as oil-based cosmetics, and certain drugs (androgenic hormones, antiepileptics, progestins (as in oral contraceptives), systemic corticosteroids, and iodide and bromide containing agents. There are also endocrine

conditions whereby there is a hypersecretion of androgen, e.g. polycystic ovarian disease, ovarian tumors, or enzymatic hyperactivity for the production of androgens or reduced metabolism of androgens.

Treatment of acne is aimed at one or more of these three causes: topical agents that remove the comedones such as benzoyl peroxide, topical vitamin A preparations enhancing flow of sebum to the surface, and oral 13-*cis*-retinoic acid can decrease sebaceous gland secretion and gland size. Oral vitamin A preparations are known teratogens and should be avoided in patients who are or plan to become pregnant.

Table 54 lists some current candidate therapeutic interventions in development for the treatment of acne and related skin disorders.

I. Alopecia

Under normal conditions, scalp hair grows between 10-15mm each month.

Under normal conditions, 80-85% of hair-follicles are in the growing anagen stage, and 15-20% are in the dormant or telogen stage. There are multiple factors that affect the transition of the active to dormant stages and vice versa as well as factors that can affect the rate of growth and condition of hair, including physical, chemical, and emotional events. If severe conditions exist, hair growth can completely stop leading to local or wide spread hair loss. There are two types of hair loss, nonscarring (reversible) and scarring (irreversible).

Nonscarring or localized hair loss includes alopecia areata, tinea capitis, trichotillomania, androgenic alopecia, or traction alopecia. Localized hair loss is characterized by well-circumscribed, round, or oval patches of nonscarring hair loss which usually occurs on the scalp, eyelashes, or eyebrows. Patterns and location of hair loss can define whether there is a poor prognosis for return of hair growth.

Alopecia areata may be autoimmune disease and is associated with cases of Hashimoto's thyroiditis, and pernicious anemia; alopecia areata is treated with glucocorticoid topical preparations. Tinea capitis is an infection predominantly with *Trichophyton tonsurans* and is treated with griseofulvin. Trichotillomania is a disorder referring to traumatic, self-induced alopecia and usually results from persistent twisting, rubbing and pulling resulting in localized hair loss and is treated with emotional or psychiatric therapy. Androgenic alopecia is the familiar male pattern baldness that occurs slowly as a thinning of the hair shafts and eventual loss. Androgenic alopecia is genetically predetermined and is dependent on androgens. Traction alopecia occurs in subjects that over use or abuse hair styling, curling, or other traumatic devices or procedures that damage hair to the extent of hair loss. Hair loss can be further associated with secondary syphilis.

Diffuse or generalized hair loss can occur as a result of a disruption of the normal hair growth cycle. In these cases, full loss of scalp hair may be caused by severe psychological or emotional stress, systemic illness, major surgery with general anesthesia, amphetamines, β -blockers, lithium, probenecid, pregnancy, or discontinuation of oral contraceptives. Disruption of the anagen phase via one or more of these hair growth toxicities may weaken the hair shaft and hair breaks easily. For example, cytotoxic cancer chemotherapeutic agents and radiotherapy to the scalp affect the anagen hair growth phase. Retinoids and hypervitaminosis interferes with the keratinization of the hair shaft. Diffuse hair loss may occur in cases of hyperthyroidism and nutritional deficiency.

Seborrheic dermatitis appears as erythema and yellow greasy scales throughout the scalp may be associated with mild diffuse hair loss.

Lastly, scarring alopecia may be the result of systemic lupus erythematosus, discoid lupus erythematosus, morphea, and aplasia cutis.

In all cases of alopecia, removal or cessation of trauma, agents or procedures that are damaging to the hair follicles or shafts is the first line of therapy. Further, glucocorticoids topical agents can be used to reduce inflammatory or autoimmune components of the localized or diffuse hair loss. Topical Minoxidil, for the treatment of male pattern baldness, has shown to effective in only 30% of the cases.

The androgen receptor is encoded by a gene that is known to have a region of polyglutamine repeats (encoded by CAG repeats) in the amino terminal that is responsible for transcriptional activation. In humans, the number of these CAG repeats is polymorphic. Since androgens can be important in acne, hirsutism, and androgenetic alopecia (AGA), a recent study set out to determine whether these polymorphic repeats were associated with the signs and symptoms of these clinical disorders (Sawaya and Shalita, J Cutan Med Surg 3(1):9-15, 1998). The investigators found that normal subjects had a mean of 22 ± 4 ($n=48$) and 21 ± 3 ($n=60$) CAG repeats in this region of their androgen receptor for men and women, respectively. In contrast, men with AGA had 19 ± 3 and women with AGA had 17 ± 3 CAG repeats. These data are suggestive that CAG repeat length found in a physiologic relevant site in the androgen receptor may be indicative of the role androgens play in the mediation of androgenetic alopecia.

Table 55 lists the current agents, drugs, or candidate therapeutic interventions that are in development of the therapy of alopecia.

J. Adrenal dysfunction

The major function of the adrenal cortex is to produce glucocorticoids (cortisol) and mineralocorticoids (aldosterone). Either an excess or deficiency in

adrenal cortical hormones can have major physiologic effects. Cortisol is responsible for the regulation of carbohydrate metabolism, intermediate metabolism, hemodynamic functions, and developmental processes. Excess cortisol is termed Cushing's disease and cortisol deficiency is termed Addison's disease. Aldosterone is a hormone primarily involved in the regulation sodium, potassium, and hydrogen ion balance and secondarily in the regulation of blood pressure. Hyperaldosteronism or hypoaldosteronism are the terms for excess or deficiency of aldosterone. Besides cortisol and aldosterone, there are many other steroids produced in the adrenal cortex; in females the adrenal cortex is the major source of androgens.

The biosynthetic steps for the production of steroids compounds in the adrenal cortex proceeds via a series of enzymatic steps, the first molecule to enter the cycle is cholesterol, intermediates steroids (including DHEA sulfate, 17 α -OH-progesterone, 11-deoxycortisone, testosterone, androstenediones, deoxycortisols, corticosterones), and final products estradiol-17 β (E₂), estrone (E₁), cortisol, and aldosterone. Under normal conditions, cortisol is the major end-product with aldosterone next, and very little estradiol or estrone.

Adrenal cortical steroids are secreted in response to adrenocorticotrophic hormone that is secreted from the pituitary in response to stimulation by corticotropin releasing hormone secreted by the hypothalamus. There is a negative feed back loop, in that cortisol inhibits the secretion of ACTH and CRH at the pituitary and the hypothalamus, as well as somatostatin acting in the same manner as cortisol to attenuate secretion of the hypothalamus and pituitary hormones.

Once secreted, cortisol is approximately 90-93% bound by plasma proteins; albumin and the major protein being corticosteroid binding protein (CBG, transcortin). CBG has a high affinity for cortisol and is not required for transport, nor cortisol function. CBG is produced in the liver and the concentrations found in plasma is genetically determined and is regulated by hormone levels. CBG levels are increased during certain physiological conditions including pregnancy, hyperthyroidism, diabetes, in excess estrogen, and during the administration of oral contraceptives. CBG levels can be low or deficient during periods of malnutrition, in liver disease, multiple myeloma, obesity, hypothyroidism, and part of the nephrotic syndrome. In cases whereby there is an increase or decrease in the levels of CBG, bound cortisol levels increase or decrease, respectively, however there is a constant level of free cortisol. Mineralocorticoids, once secreted, are approximately 60% bound to plasma albumin.

Nearly 99% of the adrenal cortical steroids are metabolized prior to excretion. Thus, any defect or dysfunction in the enzymes involved or in the metabolic rates can result in elevated levels of cortisol or active metabolites.

Further, metabolic enzymatic reactions occur to ensure that products are sufficiently different to not elicit a biological effect in the metabolizing organ. For example, the 11 β -hydroxyl group of cortisol can be metabolized in the liver to the ketone form which is devoid of cortisol receptor binding activity. Conversely, cortisol in the kidney can be metabolized to cortisone which prevents cortisol from binding to the mineralocorticoid receptor in the kidney. Cortisol and aldosterone are cleared from the plasma with a half-lives of 80-120 minutes and 15 minutes, respectively. The changes of metabolic rates can occur via 1) inhibitory influences of plasma binding on clearance rates, 2) enhanced metabolic enzymatic activity. The metabolism of these steroid hormones can be altered by: 1) decreased metabolism, or 2) increased metabolism. Glycyrrhetic acid, present in licorice, and carbenoxolone block the 11 β -hydroxysteroid dehydrogenase activity and thereby prevent the conversion of cortisol to cortisone. Thus alterations as described above can lead to enhanced or decreased adrenal cortical steroid hormone activity and physiologic response.

Nearly 80% of the primary adrenocortical insufficiency cases are due to autoimmune destruction of the adrenal cortical tissue. Autoimmune adrenocortical insufficiency has some genetic predisposition; 40% of the cases have first or second degree relatives with similar clinical patterns. Nearly all the cases of secondary adrenocortical insufficiency is the result of limited secretion of ACTH.

Therapy of adrenocortical insufficiency is treated in the acute setting with intravenous soluble steroids and control of fluid and electrolyte balance. For the maintenance of cortisol levels, these patients are put on a schedule of cortisol administrations that mimic the normal physiologic circadian rhythm.

Hypersecretion of cortisol is termed Cushing's syndrome may be caused by adenocortical tumors hypersecreting cortisol, conditions that increase ACTH secretion, and by prolonged administration of corticosteroids. This syndrome is characterized by a moon face, increased fat pads, red cheeks, pedulous abdomen, abdominal striae, poor muscle development, poor wound healing, and bruisability with ecchymoses. Therapy of Cushing's syndrome is dependent on the etiology of the disease. Adrenocortical and pituitary tumors can be surgically removed, however in each case disruption of normal glandular function must be avoided. Bilateral removal of adrenal glands can lead to Nelson's syndrome which is thought to arise due to the loss of cortisol negative feedback on the pituitary gland. In the absence of tumors, drugs may be used to limit the secretion of ACTH or cortisol

they include: reserpine, bromocriptine, cyproheptadine, and valproate sodium can be used to reduce the secretion of ACTH, however only a minority of patients respond. Ketoconazole inhibits cortisol secretion.

5 Cortisol and the many synthetic congeners are the mainstay drug or therapy for many inflammatory diseases, conditions, or disorders and in the transplantation setting. Corticosteroids affect the immune response by decreasing growth and development of mast cells, inducing apoptosis, suppressing lymphocyte generation of IL-5 and other cytokines, inhibiting some mediator release, inhibiting cytokine production, inhibiting the transcription of cytokines (for example IL-8, TNF- α ,
10 prototypic antiviral chemokine (regulated-on-activation normal T-expressed and secreted, RANTES), and GM-CSF), and inhibiting nitric oxide synthesis. As well as providing antiinflammatory therapy, corticosteroids suppress immune function by inhibiting the activation of T cells. Steroids are highly effective in the early induction and maintenance regimens and are first line therapy in acute allograft
15 rejection.

Glucocorticoid associated side effects include increased appetite, weight gain, fluid retention, acne, ecchymosis, development of cushoid facies, hypertension, hyperkalemia, diabetes, hyperglycemia, hyperosmolar state, hyperlipidemia, hepatic steatosis, atherosclerosis, myopathy, aseptic necrosis, osteoporosis, ulcers,
20 pancreatitis, pseudotumor cerebri, psychosis, glaucoma, cataract formation, vascular necrosis, increased susceptibility to infection, impairment of the hypothalamus-pituitary axis, decreased thyroid hormone serum binding proteins, and impaired wound healing.

Mineralocorticoid hypersecretion occurs due to adrenocortical adenoma,
25 bilateral adrenocortical hyperplasia, and adrenal carcinoma. Clinically, the symptoms include hypertension, suppression of plasma renin, hypokalemia and associated disorders or syndromes related to each of these dysfunctions. Therapy for these conditions usually entails uni- or bilateral surgical removal of the adrenal adenoma or hyperplasia. In these cases, cortisol maintenance therapy is initiated as
30 described above.

Mineralocorticoid hyposecretion is treated with supplemental mineralocorticoid therapy.

K. *Thyroid dysfunction*

35 The thyroid gland secretes thyroxine (3, 5, 3', 5'-tetraiodothyronine, T₄) and 3, 5, 3'-triiodothyronine (T₃). The principal role for these two hormones is to regulate tissue metabolism and, in infants and young children, to regulate growth, development, and maturation of the nervous system and bone and joints. The

enzymatic pathway for the generation of T_4 and T_3 as well as the conversion of T_4 to T_3 (within the liver and the kidneys) are known and genes involved in these pathways are listed in Table 5.

5 The regulation of thyroid hormone secretion is part of the hypothalamus-pituitary axis; by which thyroid releasing hormone (TRH, secreted from the hypothalamus) acts on the pituitary gland to secrete thyroid-stimulating hormone (TSH) that acts on the thyroid gland to stimulate the secretion of T_4 and T_3 . Somatostatin, and other neuropeptides or neurotransmitters regulate the thyroid gland secretion activity by inhibiting secretion of TSH at the level of the pituitary
10 gland. T_3 can directly suppress the the level of proTRH mRNA in the paraventricular nucleus of the hypothalamus.

Circulating thyroid hormones are bound to throxine-binding globulin, transthyretin, or albumin, which are involved in the transport of the thyroid hormones to their target tissues. The concentrations of these binding proteins
15 change under various physiologic conditions and can affect the efficacy and tissue distribution of the thyroid hormones. These condidtions include 1) increased serum thyroid hormone-binding proteins: pregnancy, exposure to supraphysiologic levels of estrogen, hepatic cirrhosis or acute hepatitis, acute intermittent porphyria, exposure to heroin or methadone, and clofibrate; 2) decreased serum thyroid
20 hormone binding proteins: protein malnutrition, hepatic failure, chronic illness, nephrotic syndromes, exposure to L-asparaginase, congenital abnormality (X-linked) of the binding protein genes, exposure to androgenic steroids of pharmacologic doses of glucocorticoids.

The mechanism of action of T_3 and T_4 on the target tissues is thought to
25 occur via thyroid hormone intracellular receptors that binds the hormone ligand and via a process of entry into the nuclear compartment, the hormone-receptor complex activates DNA transcription genes having a thyroid receptor response element in the promoter region.

Dysfunction of thyroid hormone pathway is clinically expressed as either
30 hyperthyroidism or hypothyroidism. In either case, there are multiple levels of possible or potential disruptions of the thyroid hormone signalling pathway.

Hyperthyroidism or Graves' disease is also termed thyroidtoxicosis and may be associated with catecholamine excess, toxic multinodular goiter, toxic adenoma, iodide-induced hyperthyroidism, subacute thyroiditis, factitious (exogenous)
35 thyrotoxicosis, neonatal thyrotoxicosis (mother with Graves' disease), TSH-secreting pituitary tumors, nontumorigenic pituitary-induced hyperthyroidism, choriocarcinoma or hydatiform mole, struma ovarii, and hyperfunctioning thyroid carcinoma. Clinically, symptoms include marked ophthalmopathy (preorbital

swelling, exophthalmos, limitation of extraocular movements, protruding eyes and easy tearing), pretibial myxedema, tachycardia, elevated systolic blood pressure, and increased inotropic activity in the myocardium.

Therapy of hyperthyroidism follows two stages, 1) reestablishment of the euthyroidism, and 2) induction of a permanent alteration of thyroid function. In the first, reduction of elevated thyroid hormone secretion can be achieved by administration of thiourea derivatives (for example, propylthiouracil, methimazole, carbimazole). These agents inhibit the organification of iodine within the thyroid gland and suppress the production of the thyroid hormones. Side effects of these thiourea compounds include maculopapular rash, hepatocellular damage, agranulocytosis, and vasculitis. Other compounds used for the acute therapy of hyperthyroidism include lithium, iopanoic acid, and iopodate. In the second stage of therapy for hyperthyroidism, long-term therapy of propylthiouracil may induce remission of the hypersecretion. If remission is not attained, surgical removal of the thyroid gland, or treatment with ^{131}I . Unfortunately, radical therapies to remove or ablate function of the thyroid gland can lead to hypothyroidism.

In hypothyroidism, there is impaired secretion of the thyroid hormones. Hypothyroidism may be associated with acquired disease (Hashimoto's thyroiditis, idiopathic myxedema, ^{131}I radiotherapy, external radiation therapy to the neck area, subacute thyroiditis, cystinosis, impaired function of thyroid gland (iodine deficiency or excess, drug induced (lithium carbonate, para-aminosalicylic acid, thiourea drugs, sulfonamides, phenylbutazone, and others)), congenital genetic defects (biosynthetic enzymes for the thyroid hormones, thyroid agenesis, thyroid dysgenesis or ectopy, maternal iodide or antithyroid drugs), hypothalamic dysfunctions (neoplasms, eosinophilic granuloma, therapeutic irradiation), or pituitary dysfunction (neoplasms, pituitary surgery or irradiation, idiopathic hypopituitarism, Sheehan's syndrome, exposure to supraphysiologic levels of dopamine). Clinically, symptoms include weakness, fatigue, lethargy; dry, coarse skin; swelling of the hands, face and extremities; cold intolerance and decreased sweating; modest weight gain; decreased memory; hearing impairment; arthralgia and paresthesias; constipation; and muscle cramps. In infants or young children in which hypothyroidism remains unchecked during the first two years of life, irreversible mental retardation as part of a syndrome called cretinism develops.

Therapy of hypothyroidism includes the replacement of synthetic thyroid hormones, T_4 and T_3 . In these cases, hormone replacement therapy is sufficient to restore euthyroidism. Special cases of hypothyroidism, for example those individuals with angina and hypothyroidism require special monitoring since the replacement hormones may stimulate the myocardial oxygen demands in a

myocardium that can not produce adequate myocardial blood flow. Another special case are patients with severe myxedema coma, and event that may arise in patients with severe hypothyroidism and are subjected to additional physiologic stresses.

Anthithyroid antibodies can be part of an autoimmune thyroid disease, such as Hashimoto's or Graves' disease. Patients may have serum antibodies formed to thyroid peroxidase (common), serum thyroglobulin, or to the TSH receptor.

L. Parathyroid dysfunction

Parathyroid hormone is secreted by the parathyroid glands. The hormone is responsible for the regulation of bone resorption and calcium mobilization. In addition to increasing the the plasma Ca^{+} levels and depressing the plasma phosphate levels, parathyroid hormone increases the excretion of phosphate in the urine.

In cases of pseudohypoparathyroidism, patients have normal circulating levels of parathyroid hormone, but lack the GTP-binding protein to allow hormone receptor-G-protein stimulated adenylate cyclase activity and subsequent increases in intracellular cAMP. In another form of pseudohypoparathyroidism, there is an adequate GTP-binding protein, but there is lacking the intracellular messenger system to allow parathyroid hormone mediated phosphaturic action of the hormone within the target tissues. In cases of parathyroidectomy, hypocalcemia, tetanus, and hyperphosphatemia occurs. Administration of parathyroid hormone can restore calcium and phosphate ion stasis.

In cases of parathyroid hormone excess, usually a result of inordinate administration of parathyroid hormone or a tumor hypersecretion of parathyroid hormone, the symptoms include hypercalcemia, hypophosphatemia, and demineralization of the bones, and the formation of calcium containing kidney stones. Removal of the tumor or adjustment of the parathyroid hormone administration schedule is the prudent course of treatment. Secondary hyperparathyroidism may be the result of chronic renal disease.

In nearly 20% of cancer patients there is marked hypercalcemia as result of bone metastases that produce the hypercalcemia as a result of the eroding bone. The bone erosion may be the result of prostaglandin E and the tumor or cancerous cells. Further some cancers cells hypersecrete 1,25-dihydroxycholecalciferol, or another bone related hormones. In some cancers, there has been detected hypersecretion of parathyroid hormone-related protein. Tumors in this category include breast, kidney, ovary, and skin.

Although the above description includes the hypothalamus-pituitary-target gland axes, there are other organs that have endocrine functions. These include the kidneys, the heart, and the pineal gland.

The kidneys regulate blood pressure via the renin-angiotensin system. The kidneys produce and secrete renin (in the juxtaglomerular apparatus), an acid protease that acts on angiotensinogen to form angiotensin I. The next enzyme in the pathway is angiotensin converting enzyme (ACE, located in the lungs and
5 elsewhere) which converts angiotensin I to angiotensin II. Angiotensin II acts directly on vascular smooth muscle to to arteriolar constriction and leads to an increase in blood pressure, on the adrenal cortex to stimulate secretion of aldosterone, and in the cerebral cortex to decrease the baroreflex potentiation g the pressor effects. Angiotensin II is metabolized by various peptidases
10 (aminopeptidase) and is sequestered in vascular beds of tissues by as yet unknown molecule trapping mechanism.

ACE, angiotensin and renin receptors, and regulation of renin secretion have proven excellent candidate targets for drug intervention for the treatment of hypertension and other cardiovascular disease. Other likely candidates for the
15 therapuetic intervention of the renin-angiotensin system are listed in Table 5 and Table 11.

The kidneys, and to a lesser extent the liver, also produce and secrete erythropoietin. In adults, erythropoietin is produced by the intersitial cells in the peritubular capillary bed of the kidneys and the perivenous hepatocytes in the liver.
20 Erythropoietin regulates the production of erythrocytes by stimultating the munber of erythropoietin-sensitive committed stem cells in the bone marrow that are converted to precursors and ultimately to mature erythrocytes. When erythropoietin levels are low, erythroid stem cells show DNA cleavage followed by programmed cell death (apoptosis). Erythropoietin reduces the DNA cleavage and stimulates the
25 cells to survive. When the renal mass is reduced in adults by renal disease or nephrectomy, the resultant reduction in the production of erythropoietin, and the inability of the liver production to compensate for this reduction, leads to marked anemia. Synthetic or recombinant erythropoietin has proven to be therpauetically improtant to those individuals in end-stage renal disease and other anemic conditions
30 such as cancer, trauma, surgery, and others. Other genes involved in the erythrpoeitin pathway are listed in Table 5.

The myocardium produces and secretes atrial natriuretic peptide (ANP). ANP produces natriuresis, in part by stimulating an increase in glomular filtration rate, promotes tubule secretion of sodium, and lowers blood pressure by acting
35 directly on the vascular smooth muscle cells and descreasin rhe responsiveness to pressor substances. In the brain, ANP actions are opposite of those directed by angiotensin II. ANP is metabolized by neutral endopeptidase (inhibited by thiorphan) and has a short half-life.

The other endocrine hormone involved in natriuresis is produced and secreted from the adrenal glands and is termed the Na⁺/K⁺ ATPase inhibiting factor. This factor produces natriuresis by inhibiting the Na⁺/K⁺ ATPase and produces an increase in blood pressure.

5 The pineal gland produces and secretes melatonin. In humans, melatonin is produced and secreted during the dark periods of the day and is maintained at lower concentrations during the daylight hours. Melatonin has been implicated in inducing and maintaining sleep. Melatonin is synthesized from serotonin via two enzymes found in the pineal parenchymal cells. Melatonin is secreted via a neural
10 stimulation to the pineal gland. β -Adrenergic stimulation to the pineal gland results in increased stimulation of the production and secretion of melatonin. Metabolism of melatonin occurs via 6-hydroxylation followed by conjugation in the liver and is predominantly excreted in the urine.

Cardiovascular and Renal Disease

15 There are some examples whereby there is no direct evidence that a gene or genes are involved in drug response of a candidate therapeutic intervention. In these cases, however, there is genetic data supporting a role of a variance or variances involved in the etiology, progression, or risk of a cardiovascular or renal disease. These cases, including but not limited to are described below with details of current
20 therapies and potential genetic involvement of variances in drug responses.

A. Anemia

Anemia is a condition in which the number of red blood cells per cubic mm, the amount of hemoglobin in 100 ml of blood, and the volume of packed red cells
25 per 100 ml of blood are less than normal values. Anemia may be clinically manifested as pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, shortness of breath, lethargy, and fatigability or other signs and symptoms. Anemia can be caused by three broad defects 1) bone marrow failure, 2) acute blood loss, and 3) hemolysis, however,
30 anemia may be the result of one or more of these three. Anemia is a common manifestation of many different chronic or acute diseases, toxins, therapeutic drugs, nutritional status, endocrine disorders, congenital conditions, autoimmune conditions, alcohol, drug, or substance abuse, trauma, surgery, or any other condition that affects the function or status of the bone marrow, blood volume, or
35 erythrocytes. When anemia develops, there are compensatory physiological mechanisms that are available to attempt to restore tissue oxygenation including

increases in the erythrocyte glycolytic intermediate 2,3-diphosphoglycerate (2,3-DPG; binds to hemoglobin and decreases the oxygen binding affinity) in erythrocytes, increased peripheral dilation, increased cardiac stroke volume, decrease in blood pressure, or other mechanisms.

5 Anemia may be due to drug toxicities. Aplastic anemia or hematologic blood disorders may also be due to a proliferative defect and related bone marrow failure syndromes.

 Anemia due to bone marrow failure usually results in changes in mean cell volume (MCV) can be categorized as normocytic, microcytic, and macrocytic
10 anemia. Normocytic bone marrow failure can be the result of iron deficiency, chronic disease, renal failure, liver disease, endocrine disorders, aplasia, myelodysplasias, myelofibrosis, hematologic or solid tumors, granulomas, human immunodeficiency virus (HIV) infection, and others. Microcytic bone marrow
15 failure can be the result of iron deficiency, chronic disease, thalassemias, aluminum toxicity, thyrotoxicosis, hereditary sideroblastic conditions and others. Macrocytic bone marrow failure can be the result of megaloblastic conditions (cobalamin and folate deficiencies, and congenital disorders), alcoholism, drugs, liver disease,
20 aplasia, myelodysplasias, myelofibrosis, hematologica or solid tumors, granulomas, human immunodeficiency virus (HIV) infection, hypothyroidism, splenectomy, and others.

 Hemolytic anemia primarily due to the destruction of red cells can be the result of congenital conditions (enzyme deficiency, membrane skeletal protein abnormalities, hemoglobinopathies) or acquired conditions (antibody-induced, mechanical fragmentation, and membrane protein anchoring abnormalities). Acute
25 blood loss occurring in trauma, surgery, or acute or chronic disease can lead to excessive blood loss.

 Drugs or other agents known to cause anemia include cancer chemotherapeutic agents (antimetabolites, alkylating agents, hydroxyurea, cytosine arabinoside and others), anti-inflammatory agents (aspirin, non-steroid anti-
30 inflammatory agents, phenylbutazone, gold compounds), antibiotics (chloramphenicol, penicillin, cephalosporins, sulfonamides and others), anticonvulsants (phenytoin and others), dihydrofolate reductase inhibitors (methotrexate, pyrimethamine, trimethoprim, triamterene, pentamidene, and others), antiviral agents (zidovudine and others), immunosuppressive agents (azathioprim
35 and others), antiarrhythmic agents (procainamide, quinidine and others), antihypertensive agents (alpha-methyldopa), antimalarials (primaquine and others), and the anticoagulants (warfarin and heparin and others).

Therapy of anemia includes blood transfusion, removal of the agent or toxin causing the anemia, or treating the underlying cause of the anemia. In some cases of anemia, erythropoietin can be used to stimulate the erythrocyte precursor cells in the bone marrow cells to produce mature erythrocytes.

5 A gene, genes, or gene pathway involved in the etiology of anemia or associated disorders or potential sites for targeted drug therapy of anemia are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of anemia are listed in Table 57.

10 *B. Angina*

Angina pectoris is a common clinical manifestation of coronary artery disease. Angina is a clinical syndrome including chest pain or discomfort brought on by exertional or anxiety, typically lasting several minutes. Patients with angina are at increased risk of myocardial infarction heart failure and death. Angina is a symptom of myocardial ischemia that is the result of myocardial oxygen demand not met by myocardial oxygen supply (for more details see below under *Ischemia*). Although the most common cause of myocardial ischemia is atherosclerotic coronary artery disease, there are other factors that may lead to this clinical syndrome, including thromboembolic disease and vasospasm. Factors related to myocardial oxygen demand include heart rate, contractility, and wall tension (ventricular volume and ventricular pressure). Unstable angina refers to angina of which occurs at rest or without a specific (exertional or environmental) trigger. Stable angina refers to predictable, event-induced chest pain. Unstable angina has been correlated with progression to acute myocardial infarction in 20% of the cases. More than 50% of the patients with unstable angina have multi-vessel disease with eccentric, irregular, or ulcerated atherosclerotic lesions associated with endothelial disruption and adherent thrombus.

Another form of angina is variant angina which is characterized by chest pain accompanied by a transient ST-segment changes (either ST elevation or depression) and ventricular arrhythmias.

Angina can often be controlled by nitrates, β -adrenergic blockers, calcium channel blockers, antiplatelet and antithrombin therapy, or combination thereof.

35 Genes, and gene pathway involved in the etiology of angina and associated disorders or potential sites for targeted drug therapy of angina are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of anemia are listed in Table 58.

C. Arrhythmia

Cardiac arrhythmias occur as a result of abnormalities of impulse generation, impulse conduction, and combined abnormalities of impulse generation and conduction. Some cardiac arrhythmias may lead to asymptomatic conditions, others lead to clinical symptoms and may be life-threatening. Abnormalities of impulse generation includes abnormal automaticity (abnormal pacemakers), triggered activity as a result of early or delayed after-depolarizations. In both alterations of automaticity and triggered activity, generation of impulses in fibers that are normally incapable of normal automaticity, e.g. atrial and ventricular tissue, ensues. Within the myocardium the conduction system can become a cardiac pacemaker. For example, the atrioventricular (AV) node.

Abnormalities of impulse conduction occurs via a process called reentry. In reentry, there occurs an area or region that is slow or unable to conduct electrical signals. This defect in conduction permits a wave of excitation to propagate continuously within a closed circuit. In these cases, the surrounding tissue is not at the same pace as the surrounding tissue and the electrical impulse passes through the normal tissue and can spread in a multi-directional manner which leads to marked asynchrony.

Heart block is the condition whereby the conduction from the atria to the ventricles is interrupted. Myocardial disease may decrease or stop conduction in one or more regions. Heart block may be complete, incomplete, include a right- or left bundle branch block, hemiblock or fascicular blocks.

Ectopic foci of excitation occurs when there is myocardial disease that renders the His-Purkinje fibers or other fibers to discharge electrical activity spontaneously. This condition leads to increased automaticity, potentially leading to extrasystole, premature beats, atrial or ventricular or nodal paroxysmal tachycardia, or atrial flutter.

Arrhythmias may also be localized to the atrial or ventricular regions. Atrial arrhythmias include atrial tachycardia, or paroxysmal atrial tachycardia with block, atrial flutter, or atrial fibrillation. Ventricular arrhythmias can include all of the previous described types of arrhythmias but also include paroxysmal ventricular tachyarrhythmia as well and ventricular fibrillation.

Accelerated AV conduction (Wolff-Parkinson-White syndrome) or the Lown-Ganong-Levine syndrome are examples of other arrhythmias that are characterized by specific electrocardiogram abnormalities.

Therapy for arrhythmias includes an understanding of the type, underlying mechanism, and treatment targeted to restore normal cardiac function. However, in some cases, mechanisms can only be inferred and therapy is based on empirical

knowledge. Current antiarrhythmic drugs can be classified as the following broad categories: Na⁺ channel blockers, K⁺ channel blockers, Ca⁺ channel blockers, β -adrenergic blockers, and digitalis. In each of these categories, there is a blockade of the activity of the specific ion channel or receptor mediated activation of the myocardial activity. Digitalis is the exception, having multiple pharmacologic effects including Ca⁺ current inhibition, stimulation of vagal tone to the myocardium, and a reduction in the K⁺ currents within the atrium.

A gene, genes, or gene pathway involved in the etiology of arrhythmia or associated disorders or potential sites for targeted drug therapy of arrhythmia are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of arrhythmias are listed in Table 59.

Hypertension

Hypertension is the clinical syndrome in which there is sustained elevation of systemic arterial pressure. There may be conditions of specific arterial hypertension to specific organs, including pulmonary, renal, hepatic arterial hypertension.

Systemic hypertension is a common abnormality that can be the result of a variety of conditions including: adrenocortical disease (Conn's syndrome, aldosteronism, hypersecretion of glucocorticoids, hypersecretion of mineralocorticoids, and pseudohyperaldosteronism), pheochromocytoma, paraganglioma, renal hypertension, renal disease (glomerulonephritis, pyelonephritis, polycystic disease, Liddle's syndrome, hypokalemic nephropathy, low-renin hypotension), Narrowing of the aorta, oral contraceptives, neurovascular compression of the rostral ventrolateral medulla. However, in most cases, the etiology is unknown (termed essential hypertension).

Therapy of hypertension includes α - or β -adrenergic blockers, inhibition of the renin-angiotension system, or converting enzyme, and calcium channel blockers. In cases whereby hypertension is the result of a condition, as listed above, the primary condition is treated with ancillary antihypertensive added. Further, reduction in the intake of sodium in the diet has been shown to assist the reduction of systemic arterial pressure.

A gene, genes, or gene pathway involved in the etiology of hypertension or associated disorders or potential sites for targeted drug therapy of hypertension are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of hypertension are listed in Table 60.

E. Hypotension

Hypotension is the condition of subnormal blood pressure. Hypotension may be the result of orthostatic hypotension, anemic conditions, fulminant meningococemia or other infections, blood transfusions, trauma, traumatic brain injury, hepatic or renal failure, and drug induced.

Hypotension is currently treated with methoamine, peripheral sympathomimetics, and vasopressin. A gene, genes, or gene pathway involved in the etiology of hypotension or associated disorders or potential sites for targeted drug therapy of hypotension are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of hypotension are listed in Table 61.

F. Ischemia

Myocardial ischemia develops when the metabolic demands exceed oxygen delivery to the myocardium. Factors that influence the myocardial oxygen supply include the oxygen capacity of the blood, coronary blood flow and vascular resistance. Factors that affect myocardial oxygen demand are heart rate, contractility, and systolic wall tension. Any agent or physiologic factor that decreases myocardial oxygen supply or increases myocardial oxygen demand may potentially lead to myocardial ischemia. There are conditions that lead to myocardial ischemia including hypertension, arrhythmias, coronary artery disease, rheumatic fever, congenital heart defects, heart failure, and myocardial infarction.

The identification and extent of myocardial damage due to myocardial oxygen demand and reduced supply clinically manifests as myocardial infarction, sudden death, angina pectoris (either uncomplicated or with infarct), and coronary insufficiency.

Therapies for myocardial ischemia currently available are described within other sections of this invention and can be found under the following sections: thrombosis, angina, hypertension, arrhythmias, and heart failure. A gene, genes, or gene pathway involved in the etiology of ischemia or associated disorders or potential sites for targeted drug therapy of ischemia are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of myocardial ischemia are listed in Tables 57, 59, 60, 62, and 64.

G. Heart Failure

Heart failure is a syndrome in ventricular dysfunction if accompanied by reduced exercise capacity. Heart failure is the final condition from a variety of cardiovascular disorders including coronary heart disease, long-standing

hypertension, valve deformities or valvular heart disease, rheumatic heart disease, nutritional cardiac disease and cardiomyopathies. Other diseases or conditions associated with heart failure include infections (systemic or cardiac specific (myocarditis), infiltrative disorders (amyloidosis, hemochromatosis, sarcoidosis), electrolyte disorders, myocardial specific toxins (substances of abuse, cancer chemotherapeutic agents), lupus erythematosus, rheumatoid arthritis, diabetes mellitus, thyroid disease, hypoparathyroidism, pheochromocytoma, and sustained or prolonged tachycardia.

Ultimately, in the failing heart, inotropic action is compromised and the resultant loss in cardiac output renders the myocardium unable to meet the systemic and peripheral metabolic demands leading to various clinical symptoms including cardiac enlargement, weakness, edema, prolonged circulation time, hepatic enlargement, shortness of breath, sensation of suffocation, and distention of peripheral veins. Dyspnea on exertion is a prominent symptom, leading to paroxysmal, and in severe cases, frank pulmonary edema.

Physiological compensatory mechanisms of heart failure can be broadly described as increased heart rate, increased preload and afterload, and cardiac hypertrophy. Each of these physiological changes are attempts to increase cardiac output which is dependent on heart rate, blood pressure and contractility.

Although the most common form of heart failure is left ventricular failure (70-90%), there are conditions whereby diastolic dysfunction occurs (10-30%). Clinically these two are treated differently. For left ventricular (LV) failure, the current therapies include a combination of antihypertensives (ACE inhibitors), diuretics, and positive inotropic agents. Refractory cases of LV failure, additional diuretics, vasodilators, and β -adrenergic blockers are added to the regimen. In diastolic dysfunction leading to failure Ca^{++} channel blockers are the first line of therapy with ACE inhibitors and β -adrenergic blockers added in refractory cases.

Heart failure is further associated with a variety of co-morbidities that can worsen the condition and prognosis including septicemia, hypo-osmolality, primary thrombocytopenia, renal hypertension disorder, myocardial infarction, pulmonary embolism, arrhythmias, intracerebral or subdural hemorrhage, cerebral thrombosis, hypotension, pneumonia, chronic renal failure, and decubitus ulcers.

A gene, genes, or gene pathway involved in the etiology of heart failure or associated disorders or potential sites for targeted drug therapy of heart failure are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of heart failure are listed

in Table 11 and complications associated with heart failure in Tables 59, 60, 62, and 64.

H. Thrombosis

5 Thrombosis is the formation of a blood clot in a blood vessel. If the thrombotic clot is large enough it may occlude the vessel and create tissue hypoxia. If unchecked, thrombosis can be a major medical problem and is associated with vessels that have sluggish blood flow, including in veins of extremities after surgery or delivery, conditions of reduced cardiac output, or in coronary or cerebral arteries
10 where the intima is damaged by atherosclerotic plaques (see below) or damage to the endocardium. Areas of thrombi have a tendency to break off from a vessel wall and can travel to distant sites, termed emboli, and create damage to other organs.

 The activation of coagulation occurs via a coordinated process of clotting factors leading to the formation of thrombin which then activates the conversion of
15 fibrinogen to fibrin and clot formation ensues. However, in the endothelial cell, when thrombin binds to thrombomodulin, thrombin has anticoagulant activity by first activating protein C. Activated protein C then inactivates an inhibitor of tissue plasminogen activator and conversion of plasminogen to plasmin occurs. Plasminogen is converted to active plasmin when tissue plasminogen activator
20 hydrolyzes the bond between arg560 and val561. Plasmin is responsible for the enzymatic breakdown of clots.

 Atherosclerosis is a complex combination of hyperlipidemia, injury to the endothelium, and inflammation. The interaction of these multiple processes in association with local genetic and hemodynamic influences may promote the
25 formation of atheromatous plaques as a reparative response of the arterial wall. Atherosclerotic plaques are composed of thrombogenic lipid-rich core protected by a fibrous cap comprising smooth muscle cells and inflammatory cells. The inflammatory cells are predominantly macrophages. As atherosclerotic plaques build blood flow is reduced creating ischemia in tissues down stream from the area
30 of the plaque.

 In another model, the stenosis created by the plaques may be a part of the resulting ischemic event. Frequently, less obstructive but more vulnerable plaques occur which are characterized by a thinned fibrous cap, marked lipid accumulation, a large number of macrophages, and a smaller amount of smooth muscle cells. It
35 has been proposed that since these plaques are more prone to rupture creating contact with the highly thrombogenic materials of the lipid-rich nucleus of these lesions, thrombosis is stimulated.

Advanced atherosclerotic lesions are caused by a series of cellular and molecular events involving replication of smooth muscle cells and macrophages on the vessel wall. The interaction of these cells with the T lymphocytes can lead to a fibroproliferative response. Large amounts of connective tissue produced by these smooth muscle cells consist of macrophages, T lymphocytes, smooth muscle cells, connective tissue, necrotic residues, and varying amounts of lipids and lipoproteins.

Endothelial cells maintain the vessel surface in a non-thrombogenic state, preventing platelet and leukocyte adhesion, and act in maintaining the vascular tonus by releasing nitric oxide, prostaglandin, and endothelin. These cells also produce growth factors, cytokines, and chemokines to maintain the integrity of the collagen- and proteoglycan-rich basement membrane. Changes in some of these functions may trigger cell interactions with monocytes, platelets, smooth muscle cells, and lymphocytes. Hyperlipidemia and hypercholesterolemia are sufficient to induce dysfunction of the endothelial modulation of the vasoactive reactions and arteriolar tonus.

Anticlotting therapy includes heparin, streptokinase, urokinase-type plasminogen activator, and tissue-plasminogen activator. Coumarin derivatives such as dicumarol and warfarin can also be effective anticoagulants. These compounds inhibit the action of vitamin K which is a necessary cofactor for the enzyme that converts glutamic acid residues to γ -carboxyglutamic acid residues. This mechanism affects the clotting factors II, VII, IX, and X, as well as protein C and protein S.

A gene, genes, or gene pathway involved in the etiology of thrombosis or associated disorders or potential sites for targeted drug therapy of thrombosis are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of thrombosis are listed in Table 64.

I. Renal Disease

The kidneys are primarily involved in regulating body fluid volume and composition by forming urine. The purpose of urine excretion, composed of ionic solutes, is to remove or eliminate metabolic end-products and maintain fluid volume and composition for the sustenance of physiologic function of the rest of the body. Urine formation and composition is affected by dietary intake of solutes and water as well as endogenous and exogenous carbohydrates, proteins, and nucleic acids. The kidneys also provide the mechanism to excrete drugs, toxins, and other exogenous substances.

Urine formation occurs via a sequence of five steps: 1) the glomerulus filters extracellular fluid across the glomerulus capillaries and the visceral epithelium of Bowman's capsule; the driving force is mean arterial blood pressure; 2) the proximal tubule isotonicly reabsorbs approximately two-thirds of the glomerular filtrate; 3) the loop of Henle dissociates the absorption of sodium and water; 4) the distal convoluted tubule primarily absorbs sodium under the influence of aldosterone and secretes protons, ammonia, and potassium; and lastly, 5) the collecting duct system regulates the osmolarity of urine under the influence of antidiuretic hormone. In addition to its function of producing urine, the kidney can also serve as an endocrine organ producing and secreting prostaglandins, kallikrein-kinins, erythropoietin, and renin. The kidney also has a function and role in metabolism. The kidney is a target organ for many hormones including parathyroid hormone, aldosterone, and antidiuretic hormone.

Renal dysfunction or disorders often are clinically nonspecific and are characterized by hematuria, azotemia, hypertension, and metabolic acidosis. Broadly, kidney dysfunction can be categorized as underperfusion syndromes, renal parenchymal syndromes, and post-renal syndromes.

Renal underperfusion syndromes include reduced effective circulating volume (including circulatory collapse, congestive heart failure, and cirrhosis of the liver), occlusive renal artery disease (including renal artery atherosclerosis, fibromuscular hyperplasia), and vasoconstriction of renal microvasculature (including acute transplant rejection, cyclosporin nephrotoxicity, and amphotericin B nephrotoxicity).

Renal parenchymal syndromes include acute hypertensive nephropathy, analgesic nephropathy, hemolytic-uremic syndrome, hypercalcemic nephropathy, interstitial nephritis, lupus nephritis, multiple myeloma, oxalate nephropathy, pyelonephritis, glomerulonephritis, renal vein thrombosis, Wegener's granulomatosis.

Renal failure, or the uremic syndrome, occurs when the functional renal mass is sufficiently reduced such that the kidney is longer able to conduct normal functions. Thus, the clinical hallmarks of this disease are related to the loss of urine formation, excretion, and aberrant composition of body fluids as well as loss of erythropoietin and renin and may be treated separately. These related disorders include electrolyte disorders (accumulation of potassium, sodium, phosphate, magnesium and aluminum and hypocalcemia), cardiovascular abnormalities (including accelerated atherosclerosis, hypertension, pericarditis, myocardial dysfunction), hematologic dysfunction (including anemia, leukocyte dysfunction, hemorrhagic diathesis), gastrointestinal disorders (including anorexia, nausea,

vomiting, gastroparesis, gastrointestinal bleeding), disorders of taste, renal osteodystrophy (including osteomalacia, osteitis fibrosa, osteosclerosis, osteoporosis), neurologic abnormalities (including insomnia, fatigue, psychological symptoms, asterixis, peripheral neuropathies), myopathy, impaired carbohydrate
5 intolerance (peripheral resistance to insulin)), endocrine and metabolic disorders (including glucose intolerance, insulin resistance, insulin degradation, hypoglycemia, fertility disorders, hypothermia), hyperuricemia, and pruritis, soft tissue calcification and uremic frost. In chronic renal failure, the loss of renal
10 renal function. These adaptive processes include increased glomerular filtration rate of the intact nephrons, and increased phosphate excretion. Unfortunately, as the kidney disease and the loss of renal function progresses, these adaptive processes may ultimately create more damage than restore function.

In any of the cases for renal disease there are aggravating factors that can
15 affect the progression of the disease including vascular volume depletion (as a result of diuretics, gastrointestinal fluid losses, dehydration, low cardiac output, renal hypoperfusion, atheroembolic disease, ascites, nephrotic syndrome), drugs
(including aminoglycosides, prostaglandin synthesis inhibitors, diuretics),
20 obstructions (including tubule obstruction via uric acid or Bence Jones protein or posttubular obstruction via prostatic hypertrophy, necrotic papillae, or uretal stones), infections, toxins (including radiographic contract materials), hypertensive crisis, and hypercalcemia or hyperphosphatemia.

Treatments of renal disease are dependent on whether there is an acute or
25 chronic condition. In the acute conditions, stabilization of fluid and electrolyte balance is critical for the sustenance of life. In chronic end-stage failure the patient may have to depend on exogenous dialysis or transplantation.

A gene, genes, or gene pathway involved in the etiology of renal disease or associated disorders or potential sites for targeted drug therapy of renal disease or associated disorders are depicted in Table 11 with the specific gene list in Table 6.
30 Current candidate therapeutic interventions in development for the treatment of anemia are listed in Table 57, for renal disease in Table 65, and for nephritis in Table 66.

J. Restenosis

35 Interventional cardiology includes procedures aimed at mechanically improving coronary blood flow. These procedures include intracoronary stents, coronary artery bypass surgery, and percutaneous transluminal coronary angioplasty (PCTA). Although successful resolution of coronary arterial vessel occlusion has

been accomplished with PCTA in as many as two thirds of the patients, currently nearly 20-30% of the patients require emergency bypass surgery, there is an associated 4-10% mortality, 2-5% sustain damage to the vessel including dissection, intimal disruption, perforation, and embolism, and 9% experience Q-wave
5 infarctions. Another PCTA related complication is coronary restenosis. Restenosis, or reocclusion of the coronary vessel, has predisposing factors including male gender, continued smoking after PCTA, diabetes mellitus, elevated insulin levels, absence or previous myocardial infarction, and unstable angina.

A gene, genes, or gene pathway involved in the etiology of restenosis or
10 associated disorders or potential sites for targeted drug therapy of restenosis are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of restenosis are listed in Table 67.

15 *K. Peripheral vascular disease*

Peripheral vascular disease (PVD) refers to diseases of any of the blood vessels outside the myocardium and to diseases of the lymph vessels. The disorder is often a narrowing of the blood vessels that carry blood to the arms and legs. There are two types of PVD, functional PVD and organic PVD. Functional PVD is
20 not organic and does not involve defects in the structure of the blood vessels. Functional PVD includes Raynaud's syndrome. Organic PVD are caused by structural changes in the vessel, such as inflammation and tissue damage, for example Buerger's disease. PVD can result from atheromatous narrowing of the arteries to the legs. Symptoms may range from calf pain on exercise "intermittent claudication", to rest pain and gangrene. Intermittent claudication is the commonest
25 symptom occurring in around up to 5% of men and 2.5% of women aged 60 or over. Peripheral vascular disease may be the result of venous stasis, anemia, dysbetalipoproteinemia, diabetes mellitus, and systemic sclerosis.

A gene, genes, or gene pathway involved in the etiology of peripheral
30 vascular disease or associated disorders or potential sites for targeted drug therapy of peripheral vascular disease are depicted in Table 11 with the specific gene list in Table 6. Current candidate therapeutic interventions in development for the treatment of peripheral vascular disease are listed in Table 68.

35 Advantages of Pharmacogenomic Clinical Development of Novel Candidate Therapeutic Interventions for the Treatment of Disease

The evidence that a variance in a gene involved in a pathway that affects drug response, indicates and supports the theory that there is a likelihood that other genes have similar qualities to various degrees. As drug research and development proceeds to identify more lead candidate therapeutic interventions for neurologic and psychiatric disease, there is possible utility in stratifying patients based upon their genotype for these yet to be correlated variances. Further, as described in the Detailed Description, methods for the identification of candidate genes and gene pathways, stratification, clinical trial design, and implementation of genotyping for appropriate medical management of a given disease is easily translated for patients with neurologic and psychiatric disease. As described below there are likely gene pathways as are those that are outlined in the gene pathway Tables 1-6 as described above and matrix Tables 7-11.

The advantages of a clinical research and drug development program that include the use of polymorphic genotyping for the stratification of patients for the appropriate selection of candidate therapeutic intervention includes 1) identification of patients that may respond earlier to therapy, 2) identification of the primary gene and relevant polymorphic variance that directly affects efficacy, safety, or both, 3) identification of pathophysiologic relevant variance or variances and potential therapies affecting those allelic genotypes or haplotypes, and 4) identification of allelic variances or haplotypes in genes that indirectly affects efficacy, safety or both.

Based upon these advantages, designing and performing a clinical trial, either prospective or retrospective, which includes a genotype stratification arm will incorporate analysis of clinical outcomes and potential genetic variation associated with those outcomes, and hypothesis testing of the statistically relevant correlation of the genotypic stratification and therapeutic benefits. If statistical relevance is detectable, these studies will be incorporated into regulatory filings. Ultimately, these clinical trial data will be considered during the approval for marketing process, as well as, incorporated into accepted medical management of the described indications.

By identifying subsets of patients diagnosed with anxiety that respond earlier to agents, optimal candidate therapeutic interventions may reduce the lag time prior to relief of psychiatric symptoms. Appropriate genotyping and correlation to dosing regimen would be beneficial to the patient, caregivers, medical personnel, and the patient's loved ones.

As an example of identification of the primary gene and relevant polymorphic variance that directly affects efficacy, safety, or both one could select a gene pathway as described in the Detailed Description, and determine the effect of

genetic polymorphism and therapy efficacy, safety, or both within that given pathway. By embarking on the previously described gene pathway approach, it is technically feasible to determine the relevant genes within such a targeted drug development program for the clinical indications described in this invention.

5 Identification of pathophysiologic relevant variance or variances and potential therapies affecting those allelic genotypes or haplotypes will speed the drug development. There is a need for therapies that are targeted to the disease and symptom management with limited or no undesirable side effects. Identification of a specific variance or variances within genes involved in the pathophysiologic
10 manifestation of anxiety and specific genetic polymorphisms of these critical genes can assist the development of novel anxiolytic agents and the identification of those patients that may best benefit from therapy of these candidate therapeutic alternatives.

By identifying allelic variances or haplotypes in genes that indirectly affects
15 efficacy, safety or both one could target specific secondary drug or agent therapeutic actions that affect the overall therapeutic action of conventional, atypical, or novel action.

In Tables 12-17 and 18-23, there is a listing of candidate genes and specific single nucleotide polymorphisms that may be critical for the identification and
20 stratification of an anxiety patient population based upon genotype. In matrix Tables 7-11 one skilled in the art would be able to identify these pathway specific genes or other genes listed in Tables 7-11 that may be involved in the manifestation of neurologic or psychiatric disease or are likely candidate targets for therapeutic approaches described in this invention.

25 A sample of therapies approved or in development for preventing or treating the progression of symptoms of cancer currently known in the art are shown in Table 24; for neurologic and psychiatric disease, Tables 25-36; for inflammation and immune disorders, Tables 38-49; for endocrine and metabolic disease, Tables 50-56; and for cardiovascular and renal disease, Tables 57-68. In these tables, the candidate
30 therapeutics were sorted and listed by mechanism of action. Further, the product name, the pharmacologic mechanism of action, chemical name (if specified), and the indication is listed as well.

Pharmacogenomics studies for these drugs, as well as other agents, drugs, compounds or candidate therapeutic interventions, could be performed by
35 identifying genes that are involved in the function of a drug including, but not limited to is absorption, distribution metabolism, or elimination, the interaction of the drug with its target as well as potential alternative targets, the response of the cell to the binding of a drug to a target, the metabolism (including synthesis,

biodistribution or elimination) of natural compounds which may alter the activity of the drug by complementary, competitive or allosteric mechanisms that potentiate or limit the effect of the drug, and genes involved in the etiology of the disease that alter its response to a particular class of therapeutic agents. It will be recognized to those skilled in the art that this broadly includes proteins involved in pharmacokinetics as well as genes involved in pharmacodynamics. This also includes genes that encode proteins homologous to the proteins believed to carry out the above functions, which are also worth evaluation as they may carry out similar functions. Together the foregoing proteins constitute the candidate genes for affecting response of a patient to the therapeutic intervention. Using the methods described above, variances in these genes can be identified, and research and clinical studies can be performed to establish an association between a drug response or toxicity and specific variances.

For each of the described disease indications one skilled in the art can identify novel candidate therapeutic interventions that may be used to treat the disease or symptoms and/or proceed with a regimen of palliative care. For compounds that have yet to achieve approval, or are still in development one skilled in the art can determine those candidate therapeutic interventions that may be of therapeutic benefit.

Exemplary compounds in development for the treatment of disease disorders or dysfunctions

There are many sources for obtaining information on drugs approved for human therapeutic use and for those compounds under clinical or preclinical investigation, as well as for compounds which have been identified as having a particular pharmacological activity. For products, which have been approved, the PDR contains a listing of the package inserts for all of the products available for human therapeutic intervention. The Merck Index can be used as an additional text to supplement information gathered on the candidate therapeutic interventions. For products that are under clinical or preclinical development, there are databases cataloging information on those candidate therapeutic interventions. Generally that information includes aspects of the drug development process, such as phase of development, identified therapeutic indications, name of manufacturer, mechanistic and pharmacological activities of the product. These databases are available for a fee, and include: PharmaProjects (<http://pjbpubs.co.uk/pharmamain2/html>) and R&D Focus (http://www.ims.global.com/products/lifecycle/r_and_d.htm). One skilled in the art can readily utilize these sources to determine appropriate candidate therapeutic intervention for the identified disease, disorder or condition.

Since there are a large number of candidate therapeutic interventions that are either approved for human therapeutic use or under clinical or preclinical investigation, one skilled in the art could search through publicly available or fee-for-access databases for interventions that may be of therapeutic benefit for a particular disease, disorder, or condition, and determine whether variances in particular genes correlate with interpatient variation in response to one or more of those therapeutic interventions. An example of the results of such searching is provided in Tables 24-68. In these tables, the disease, disorder or condition is listed. In order to generate a table or other compendium of information as listed in the table, one skilled in the art can search, for example as for Table 35, in databases for products having the indication "schizophrenia". Alternatively, one can search for alternative indications or co-morbidities, e.g., psychoses, neuroleptic, neurological to arrive at a more complete list of the available products. In the table, the candidate therapeutics were sorted and listed by pharmacologic mechanism of action (action):

Further, the product name, chemical name (if specified), as well as the indication considered for clinical development. If the candidate therapeutic interventions are approved for therapeutic use, then one skilled in the art can obtain dosing, adverse events, pharmacologic parameters (both pharmacokinetic and pharmacodynamic), and clinical data or information by referring to the PDR. If the candidate therapeutic intervention are in clinical or preclinical stages of drug development, then one skilled in the art would gather data on dosing, adverse events, pharmacologic parameters (both pharmacokinetic and pharmacodynamic), and clinical data or information for the drug or product sponsor. In both cases, selection of a candidate therapeutic intervention for retrospective or prospective pharmacogenetic clinical studies would use an analysis of the likelihood that there is a phenomenological or statistical support for the review of the data to ascertain whether the candidate therapeutic intervention (approved or in development) efficacy or safety profiles can be grouped based upon the individual's genotype or phenotype. In this way, a gene or genes selected, e.g., from a pathway involving the cellular or more broadly the pharmacological mechanism of actions, can be identified and genotyping can be performed in order to determine the allelic variance, variances, for haplotype. Further, one could group the individuals by such genetic variances and further by the therapeutic outcome determinants.

Pharmacogenomics studies for these drugs, as well as other agents, drugs, compounds or candidate therapeutic interventions, can be performed by identifying genes that are involved in the the function of a drug including, but not limited to is absorption, distribution metabolism, or elimination , the interaction of the drug with its target as well as potential alternative targets, the response of the cell to the

binding of a drug to a target, the metabolism (including synthesis, biodistribution or elimination) of natural compounds which may alter the activity of the drug by complementary, competitive or allosteric mechanisms that potentiate or limit the effect of the drug, and genes involved in the etiology of the disease that alter its response to a particular class of therapeutic agents. It will be recognized to those skilled in the art that this broadly includes proteins involved in pharmacokinetics as well as genes involved in pharmacodynamics. This also includes genes that encode proteins homologous to the proteins believed to carry out the above functions, which are also worth evaluation as they may carry out similar functions. Together the foregoing proteins constitute the candidate genes for affecting response of a patient to the therapeutic intervention. Using the methods described above, variances in these genes can be identified, and research and clinical studies can be performed to establish an association between a drug response or toxicity and specific variances.

Further, there may be genes within pathways that are either involved in metabolism of drugs, hormones, compounds, agents, or neurotransmitters or are involved in metabolism of various drugs or compounds. In Tables 1-6 and 12-23, there are listings of candidate genes and specific single nucleotide polymorphisms that may be critical for the identification and stratification of a patient population diagnosed with neurologic or psychiatric disease based upon genotype. Current pathways that may have involvement in the therapeutic benefit of described disease indications of this invention are listed as gene pathways and are listed in Tables 1-23. One skilled in the art would be able to identify these pathway specific gene or genes that may be involved in the manifestation of the described neurological or psychiatric disease, are likely candidate targets for novel therapeutic approaches, or are involved in mediating patient population differences in drug response to therapies for neurological or psychiatric disease described in the present invention.

As indicated in the Summary above, certain aspects of the present invention typically involve the following process, which need not occur separately or in the order stated. Not all of these described processes must be present in a particular method, or need be performed by a single entity or organization or person. Additionally, if certain of the information is available from other sources, that information can be utilized in the present invention. The processes are as follows:

- a) variability between patients in the response to a particular treatment is observed;
- b) at least a portion of the variable response is correlated with the presence or absence of at least one variance in at least one gene;
- c) an analytical or diagnostic test is provided to determine the presence or absence of the at least one variance in individual patients;
- d) the presence or absence of the variance or variances is used

to select a patient for a treatment or to select a treatment for a patient, or the variance information is used in other methods described herein.

A. Identification of Interpatient Variability in Response to a Treatment

5 Interpatient variability is the rule, not the exception, in clinical therapeutics. One of the best sources of information on interpatient variability is the nurses and physicians supervising the clinical trial who accumulate a body of first hand observations of physiological responses to the drug in different normal subjects or patients. Evidence of interpatient variation in response can also be measured
10 statistically, and may be best assessed by descriptive statistical measures that examine variation in response (beneficial or adverse) across a large number of subjects, including in different patient subgroups (men vs. women; whites vs. blacks; Northern Europeans vs. Southern Europeans, etc.).

In accord with the other portions of this description, the present invention concerns
15 DNA sequence variances that can affect one or more of:

- i. The susceptibility of individuals to a disease;
- ii. The course or natural history of a disease;
- iii. The response of a patient with a disease to a medical intervention, such as, for example, a drug, a biologic substance, physical energy such as radiation therapy, or
20 a specific dietary regimen. (The terms 'drug', 'compound' or 'treatment' as used herein may refer to any of the foregoing medical interventions.) The ability to predict either beneficial or detrimental responses is medically useful.

Thus variation in any of these three parameters may constitute the basis for initiating a pharmacogenetic study directed to the identification of the genetic
25 sources of interpatient variation. The effect of a DNA sequence variance or variances on disease susceptibility or natural history (i and ii, above) are of particular interest as the variances can be used to define patient subsets which behave differently in response to medical interventions such as those described in (iii). The methods of this invention are also useful in a clinical development
30 program where there is not yet evidence of interpatient variation (perhaps because the compound is just entering clinical trials) but such variation in response can be reliably anticipated. It is more economical to design pharmacogenetic studies from the beginning of a clinical development program than to start at a later stage when

the costs of any delay are likely to be high given the resources typically committed to such a program.

In other words, a variance can be useful for customizing medical therapy at least for either of two reasons. First, the variance may be associated with a specific disease subset that behaves differently with respect to one or more therapeutic interventions (i and ii above); second, the variance may affect response to a specific therapeutic intervention (iii above). Consider for exemplary purposes pharmacological therapeutic interventions. In the first case, there may be no effect of a particular gene sequence variance on the observable pharmacological action of a drug, yet the disease subsets defined by the variance or variances differ in their response to the drug because, for example, the drug acts on a pathway that is more relevant to disease pathophysiology in one variance-defined patient subset than in another variance-defined patient subset. The second type of useful gene sequence variance affects the pharmacological action of a drug or other treatment. Effects on pharmacological responses fall generally into two categories; pharmacokinetic and pharmacodynamic effects. These effects have been defined as follows in Goodman and Gilman's Pharmacologic Basis of Therapeutics (ninth edition, McGraw Hill, New York, 1986): "Pharmacokinetics" deals with the absorption, distribution, biotransformations and excretion of drugs. The study of the biochemical and physiological effects of drugs and their mechanisms of action is termed "pharmacodynamics."

Useful gene sequence variances for this invention can be described as variances which partition patients into two or more groups that respond differently to a therapy or that correlate with differences in disease susceptibility or progression, regardless of the reason for the difference, and regardless of whether the reason for the difference is known. The latter is true because it is possible, with genetic methods, to establish reliable associations even in the absence of a pathophysiological hypothesis linking a gene to a phenotype, such as a pharmacological response, disease susceptibility or disease prognosis.

B. Identification of Specific Genes and Correlation of Variances in Those Genes with Response to Treatment of Diseases or Conditions

It is useful to identify particular genes which do or are likely to mediate the efficacy or safety of a treatment method for a disease or condition, particularly in view of the large number of genes which have been identified and which continue to be identified in humans. As is further discussed in section C below, this correlation

can proceed by different paths. One exemplary method utilizes prior information on the pharmacology or pharmacokinetics or pharmacodynamics of a treatment method, e.g., the action of a drug, which indicates that a particular gene is, or is likely to be, involved in the action of the treatment method, and further suggests that variances in the gene may contribute to variable response to the treatment method. For example if a compound is known to be glucuronidated then a glucuronyltransferase is likely involved. If the compound is a phenol, the likely glucuronyltransferase is UGT1 (either the UGT1*1 or UGT1*6 transcripts, both of which catalyze the conjugation of planar phenols with glucuronic acid). Similar inferences can be made for many other biotransformation reactions.

Alternatively, if such information is not known, variances in a gene can be correlated empirically with treatment response. In this method, variances in a gene which exist in a population can be identified. The presence of the different variances or haplotypes in individuals of a study group, which is preferably representative of a population or populations of known geographic, ethnic and/or racial background, is determined. This variance information is then correlated with treatment response of the various individuals as an indication that genetic variability in the gene is at least partially responsible for differential treatment response. It may be useful to independently analyze variances in the different geographic, ethnic and/or racial groups as the presence of different genetic variances in these groups (i.e. different genetic background) may influence the effect of a specific variance. That is, there may be a gene x gene interaction involving one unstudied gene, however the indicated demographic variables may act as a surrogate for the unstudied allele. Statistical measures known to those skilled in the art are preferably used to measure the fraction of interpatient variation attributable to any one variance, or to measure the response rates in different subgroups defined genetically or defined by some combination of genetic, demographic and clinical criteria.

Useful methods for identifying genes relevant to the pharmacological action of a drug or other treatment are known to those skilled in the art, and include review of the scientific literature combined with inferential or deductive reasoning that one skilled in the art of molecular pharmacology and molecular biology would be capable of; large scale analysis of gene expression in cells treated with the drug compared to control cells; large scale analysis of the protein expression pattern in treated vs. untreated cells, or the use of techniques for identification of interacting proteins or ligand-protein interactions, such as yeast two-hybrid systems.

C. Development of a Diagnostic Test to Determine Variance Status

In accordance with the description in the Summary above, the present invention generally concerns the identification of variances in genes which are indicative of the effectiveness of a treatment in a patient. The identification of specific variances, in effect, can be used as a diagnostic or prognostic test.

5 Correlation of treatment efficacy and/or toxicity with particular genes and gene families or pathways is provided in Stanton et al., U.S. Provisional Application 60/093,484, filed July 20, 1998, entitled GENE SEQUENCE VARIANCES WITH UTILITY IN DETERMINING THE TREATMENT OF DISEASE (concerns the safety and efficacy of compounds active on folate or pyrimidine metabolism or
10 action) and Stanton, U.S. Provisional Application No. 60/121,047, filed February 22, 1999, entitled GENE SEQUENCE VARIANCES WITH UTILITY IN DETERMINING THE TREATMENT OF DISEASE (concerning Alzheimer's disease and other dementias and cognitive disorders), which are hereby incorporated by reference in their entireties including drawings.

15 Genes identified in the examples below and in the Tables can be used in the methods of the present invention. A variety of genes which the inventors realize may account for interpatient variation in response to treatments for neurological and psychiatric diseases, conditions, disorders, and/or the development of same are listed in Tables 1-6, and 12-23. Gene sequence variances in said genes are particularly
20 useful for aspects of the present invention.

Methods for diagnostic tests are well known in the art. Generally in this invention, the diagnostic test involves determining whether an individual has a variance or variant form of a gene that is involved in the disease or condition or the action of the drug or other treatment or effects of such treatment. Such a variance or
25 variant form of the gene is preferably one of several different variances or forms of the gene that have been identified within the population and are known to be present at a certain frequency. In an exemplary method, the diagnostic test involves determining the sequence of at least one variance in at least one gene after amplifying a segment of said gene using a DNA amplification method such as the
30 polymerase chain reaction (PCR). In this method DNA for analysis is obtained by amplifying a segment of DNA or RNA (generally after converting the RNA to cDNA) spanning one or more variances in the gene sequence. Preferably, the amplified segment is <500 bases in length, in an alternative embodiment the amplified segment is <100 bases in length, most preferably <45 bases in length.

35 In some cases it will be desirable to determine a haplotype instead of a genotype. In such a case the diagnostic test is performed by amplifying a segment of DNA or RNA (cDNA) spanning more than one variance in the gene sequence and

preferably maintaining the phase of the variances on each allele. The term "phase" refers to the relationship of variances on a single chromosomal copy of the gene, such as the copy transmitted from the mother (maternal copy or maternal allele) or the father (paternal copy or paternal allele). The haplotyping test may take part in two phases, where first genotyping tests at two or more variant sites reveal which sites are heterozygous in each patient or normal subject. Subsequently the phase of the two or more variant sites can be determined. In performing a haplotyping test preferably the amplified segment is >500 bases in length, more preferably it is >1,000 bases in length, and most preferably it is >2,500 bases in length. One way of preserving phase is to amplify one strand in the PCR reaction. This can be done using one or a pair of oligonucleotide primers that terminate (i.e. have a 3' end that stops) opposite the variant site, such that one primer is perfectly complementary to one variant form and the other primer is perfectly complementary to the other variant form. Other than the difference in the 3' most nucleotide the two primers are identical (forming an allelic primer pair). Only one of the allelic primers is used in any PCR reaction, depending on which strand is being amplified. The primer for the opposite strand may also be an allelic primer, or it may prime from a non-polymorphic region of the template. This method exploits the requirement of most polymerases for perfect complementarity at the 3' terminus of the primer in a primer-template complex. See, for example: Lo YM, Patel P, Newton CR, Markham AF, Fleming KA and JS Wainscoat. (1991) Direct haplotype determination by double ARMS: specificity, sensitivity and genetic applications. *Nucleic Acids Res* July 11;19(13):3561-7.

It is apparent that such diagnostic tests are performed after initial identification of variances within the gene, which allows selection of appropriate allele specific primers.

Diagnostic genetic tests useful for practicing this invention belong to two types: genotyping tests and haplotyping tests. A genotyping test simply provides the status of a variance or variances in a subject or patient. For example suppose nucleotide 150 of hypothetical gene X on an autosomal chromosome is an adenine (A) or a guanine (G) base. The possible genotypes in any individual are AA, AG or GG at nucleotide 150 of gene X.

In a haplotyping test there is at least one additional variance in gene X, say at nucleotide 810, which varies in the population as cytosine (C) or thymine (T). Thus a particular copy of gene X may have any of the following combinations of nucleotides at positions 150 and 810: 150A-810C, 150A-810T, 150G-810C or 150G-810T. Each of the four possibilities is a unique haplotype. If the two nucleotides interact in either RNA or protein, then knowing the haplotype can be

important. The point of a haplotyping test is to determine the haplotypes present in a DNA or cDNA sample (e.g. from a patient). In the example provided there are only four possible haplotypes, but, depending on the number of variances in the gene and their distribution in human populations there may be three, four, five, six or
5 more haplotypes at a given gene. The most useful haplotypes for this invention are those which occur commonly in the population being treated for a disease or condition. Preferably such haplotypes occur in at least 5% of the population, more preferably in at least 10%, still more preferably in at least 20% of the population and most preferably in at least 30% or more of the population. Conversely, when the
10 goal of a pharmacogenetic program is to identify a relatively rare population that has an adverse reaction to a treatment, the most useful haplotypes may be rare haplotypes, which may occur in less than 5%, less than 2%, or even in less than 1% of the population. One skilled in the art will recognize that the frequency of the adverse reaction provides a useful guide to the likely frequency of salient causative
15 haplotypes.

Based on the identification of variances or variant forms of a gene, a diagnostic test utilizing methods known in the art can be used to determine whether a particular form of the gene, containing specific variances or haplotypes, or
20 combinations of variances and haplotypes, is present in at least one copy, one copy, or more than one copy in an individual. Such tests are commonly performed using DNA or RNA collected from blood, cells, tissue scrapings or other cellular materials, and can be performed by a variety of methods including, but not limited to, PCR based methods, hybridization with allele-specific probes, enzymatic mutation detection, chemical cleavage of mismatches, mass spectrometry or DNA
25 sequencing, including minisequencing. Methods for haplotyping are described above. In particular embodiments, hybridization with allele specific probes can be conducted in two formats: (1) allele specific oligonucleotides bound to a solid phase (glass, silicon, nylon membranes) and the labelled sample in solution, as in many DNA chip applications, or (2) bound sample (often cloned DNA or PCR amplified
30 DNA) and labelled oligonucleotides in solution (either allele specific or short – e.g. 7mers or 8mers – so as to allow sequencing by hybridization). Preferred methods for diagnostic testing of variances are described in four patent applications Stanton et al, entitled A METHOD FOR ANALYZING POLYNUCLEOTIDES, serial numbers 09/394,467; 09/394,457; 09/394,774; and 09/394,387; all filed September 10, 1999.
35 The application of such diagnostic tests is possible after identification of variances that occur in the population. Diagnostic tests may involve a panel of variances from one or more genes, often on a solid support, which enables the simultaneous determination of more than one variance in one or more genes.

D. Use of Variance Status to Determine Treatment

The present disclosure describes exemplary gene sequence variances in genes identified in a gene table herein (e.g., Tables 12-17 and 18-23), and variant forms of these gene that may be determined using diagnostic tests. As indicated in the Summary, such a variance-based diagnostic test can be used to determine whether or not to administer a specific drug or other treatment to a patient for treatment of a disease or condition. Preferably such diagnostic tests are incorporated in texts such as are described in Clinical Diagnosis and Management by Laboratory Methods (19th Ed) by John B. Henry (Editor) W B Saunders Company, 1996; Clinical Laboratory Medicine : Clinical Application of Laboratory Data, (6th edition) by R. Ravel, Mosby-Year Book, 1995, or other medical textbooks including, without limitation, textbooks of medicine, laboratory medicine, therapeutics, pharmacy, pharmacology, nutrition, allopathic, homeopathic, and osteopathic medicine; preferably such a test is developed as a 'home brew' method by a certified diagnostic laboratory; most preferably such a diagnostic test is approved by regulatory authorities, e.g., by the U.S. Food and Drug Administration, and is incorporated in the label or insert for a therapeutic compound, as well as in the Physicians Desk Reference.

In such cases, the procedure for using the drug is restricted or limited on the basis of a diagnostic test for determining the presence of a variance or variant form of a gene. Alternatively the use of a genetic test may be advised as best medical practice, but not absolutely required, or it may be required in a subset of patients, e.g. those using one or more other drugs, or those with impaired liver or kidney function. The procedure that is dictated or recommended based on genotype may include the route of administration of the drug, the dosage form, dosage, schedule of administration or use with other drugs; any or all of these may require selecting or determination consistent with the results of the diagnostic test or a plurality of such tests. Preferably the use of such diagnostic tests to determine the procedure for administration of a drug is incorporated in a text such as those listed above, or medical textbooks, for example, textbooks of medicine, laboratory medicine, therapeutics, pharmacy, pharmacology, nutrition, allopathic, homeopathic, and osteopathic medicine. As previously stated, preferably such a diagnostic test or tests are required by regulatory authorities and are incorporated in the label or insert as well as the Physicians Desk Reference.

Variances and variant forms of genes useful in conjunction with treatment methods may be associated with the origin or the pathogenesis of a disease or condition. In many useful cases, the variant form of the gene is associated with a

specific characteristic of the disease or condition that is the target of a treatment, most preferably response to specific drugs or other treatments. Examples of diseases or conditions ameliorable by the methods of this invention are identified in the Examples and tables below; in general treatment of disease with current methods, particularly drug treatment, always involves some unknown element (involving efficacy or toxicity or both) that can be reduced by appropriate diagnostic methods.

Alternatively, the gene is involved in drug action, and the variant forms of the gene are associated with variability in the action of the drug. For example, in some cases, one variant form of the gene is associated with the action of the drug such that the drug will be effective in an individual who inherits one or two copies of that form of the gene. Alternatively, a variant form of the gene is associated with the action of the drug such that the drug will be toxic or otherwise contra-indicated in an individual who inherits one or two copies of that form of the gene.

In accord with this invention, diagnostic tests for variances and variant forms of genes as described above can be used in clinical trials to demonstrate the safety and efficacy of a drug in a specific population. As a result, in the case of drugs which show variability in patient response correlated with the presence or absence of a variance or variances, it is preferable that such drug is approved for sale or use by regulatory agencies with the recommendation or requirement that a diagnostic test be performed for a specific variance or variant form of a gene which identifies specific populations in which the drug will be safe and/or effective. For example, the drug may be approved for sale or use by regulatory agencies with the specification that a diagnostic test be performed for a specific variance or variant form of a gene which identifies specific populations in which the drug will be toxic. Thus, approved use of the drug, or the procedure for use of the drug, can be limited by a diagnostic test for such variances or variant forms of a gene; or such a diagnostic test may be considered good medical practice, but not absolutely required for use of the drug.

As indicated, diagnostic tests for variances as described in this invention may be used in clinical trials to establish the safety and efficacy of a drug. Methods for such clinical trials are described below and/or are known in the art and are described in standard textbooks. For example, diagnostic tests for a specific variance or variant form of a gene may be incorporated in the clinical trial protocol as inclusion or exclusion criteria for enrollment in the trial, to allocate certain patients to treatment or control groups within the clinical trial or to assign patients to different treatment cohorts. Alternatively, diagnostic tests for specific variances may be performed on all patients within a clinical trial, and statistical analysis performed comparing and contrasting the efficacy or safety of a drug between individuals with

different variances or variant forms of the gene or genes. Preferred embodiments involving clinical trials include the genetic stratification strategies, phases, statistical analyses, sizes, and other parameters as described herein.

5 Similarly, diagnostic tests for variances can be performed on groups of patients known to have efficacious responses to the drug to identify differences in the frequency of variances between responders and non-responders. Likewise, in other cases, diagnostic tests for variance are performed on groups of patients known to have toxic responses to the drug to identify differences in the frequency of the variance between those having adverse events and those not having adverse events.
10 Such outlier analyses may be particularly useful if a limited number of patient samples are available for analysis. It is apparent that such clinical trials can be or are performed after identifying specific variances or variant forms of the gene in the population. In defining outliers it is useful to examine the distribution of responses in the placebo group; outliers should preferably have responses that exceed in
15 magnitude the extreme responses in the placebo group.

The identification and confirmation of genetic variances is described in certain patents and patent applications. The description therein is useful in the identification of variances in the present invention. For example, a strategy for the development of anticancer agents having a high therapeutic index is described in
20 Housman, International Application PCT/US94/08473 and Housman, INHIBITORS OF ALTERNATIVE ALLELES OF GENES ENCODING PROTEINS VITAL FOR CELL VIABILITY OR CELL GROWTH AS A BASIS FOR CANCER THERAPEUTIC AGENTS, U.S. Patent 5,702,890, issued December 30, 1997, which are hereby incorporated by reference in their entireties. Also, a number of
25 gene targets and associated variances are identified in Housman et al., PCT/US98/05419, entitled TARGET ALLELES FOR ALLELE-SPECIFIC DRUGS, filed March 19, 1998, which is hereby incorporated by reference in its entirety, including drawings.

The described approach and techniques are applicable to a variety of other
30 diseases, conditions, and/or treatments and to genes associated with the etiology and pathogenesis of such other diseases and conditions and the efficacy and safety of such other treatments.

Useful variances for this invention can be described generally as variances
35 which partition patients into two or more groups that respond differently to a therapy (a therapeutic intervention), regardless of the reason for the difference, and regardless of whether the reason for the difference is known.

III. From Variance List to Clinical Trial: Identifying Genes and Gene Variances that Account for Variable Responses to Treatment

There are a variety of useful methods for identifying a subset of genes from a large set of candidate genes that should be prioritized for further investigation with respect to their influence on inter-individual variation in disease predisposition or response to a particular drug. These methods include for example, (1) searching the biomedical literature to identify genes relevant to a disease or the action of a drug, (2) screening the genes identified in step 1 for variances. A large set of exemplary variances are provided in Tables 12-23. Other methods include (3) using computational tools to predict the functional effects of variances in specific genes, (4) using *in vitro* or *in vivo* experiments to identify genes which may participate in the response to a drug or treatment, and to determine the variances which affect gene, RNA or protein function, and may therefore be important genetic variables affecting disease manifestations or drug response, and (5) retrospective or prospective clinical trials. Computational tools are described in U.S. Patent Application, Stanton et al., serial number, attorney docket number 241/034, filed April 26, 1999, entitled GENE SEQUENCE VARIANCES WITH UTILITY IN DETERMINING THE TREATMENT OF DISEASE, and in Stanton et al., Serial No. 09/419,705, filed October 14, 1999, entitled VARIANCE SCANNING METHOD FOR IDENTIFYING GENE SEQUENCE VARIANCES, which are hereby incorporated by reference in their entireties, including drawings. Other methods are considered below in some detail.

(1) To begin, one preferably identifies, for a given treatment, a set of candidate genes that are likely to affect disease phenotype or drug response. This can be accomplished most efficiently by first assembling the relevant medical, pharmacological and biological data from available sources (e.g., public databases and publications). One skilled in the art can review the literature (textbooks, monographs, journal articles) and online sources (databases) to identify genes most relevant to the action of a specific drug or other treatment, particularly with respect to its utility for treating a specific disease, as this beneficially allows the set of genes to be analyzed ultimately in clinical trials to be reduced from an initial large set. Specific strategies for conducting such searches are described below. In some instances the literature may provide adequate information to select genes to be studied in a clinical trial, but in other cases additional experimental investigations of the sort described below will be preferable to maximize the likelihood that the salient genes and variances are moved forward into clinical studies. Specific genes relevant to understanding

interpatient variation in response to treatments for major neurological and psychiatric diseases are listed in Tables 1-6. In Tables 7-11 preferred sets of genes for analysis of variable therapeutic response in specific diseases are highlighted. These genes are exemplary; they do not constitute a complete set of genes that may account for variation in clinical response. Experimental data are also useful in establishing a list of candidate genes, as described below.

(2) Having assembled a list of candidate genes generally the second step is to screen for variances in each candidate gene. Experimental and computational methods for variance detection are described in this invention, and tables of exemplary variances are provided (Tables 12-23) as well as methods for identifying additional variances and a written description of such possible additional variances in the cDNAs of genes that may affect drug action (see Stanton et al., Application No. 09/300,747, filed April 26, 1999, entitled GENE SEQUENCE VARIANCES WITH UTILITY IN DETERMINING THE TREATMENT OF DISEASE, incorporated in its entirety.

(3) Having identified variances in candidate genes the next step is to assess their likely contribution to clinical variation in patient response to therapy, preferably by using informatics-based approaches such as DNA and protein sequence analysis and protein modeling. The literature and informatics-based approaches provide the basis for prioritization of candidate genes, however it may in some cases be desirable to further narrow the list of candidate genes, or to measure experimentally the phenotype associated with specific variances or sets of variances (e.g. haplotypes).

(4) Thus, as a third step in candidate gene analysis, one skilled in the art may elect to perform *in vitro* or *in vivo* experiments to assess the functional importance of gene variances, using either biochemical or genetic tests. (Certain kinds of experiments – for example gene expression profiling and proteome analysis – may not only allow refinement of a candidate gene list but may also lead to identification of additional candidate genes.) Combination of two or all of the three above methods will provide sufficient information to narrow and prioritize the set of candidate genes and variances to a number that can be studied in a clinical trial with adequate statistical power.

(5) The fourth step is to design retrospective or prospective human clinical trials to test whether the identified allelic variance, variances, or haplotypes or combination thereof influence the efficacy or toxicity profiles for a given drug or

other therapeutic intervention. It should be recognized that this fourth step is the crucial step in producing the type of data that would justify introducing a diagnostic test for at least one variance into clinical use. Thus while each of the above four steps are useful in particular instances of the invention, this final step is indispensable. Further guidance and examples of how to perform these five steps are provided below.

- (6) A fifth (optional) step entails methods for using a genotyping test in the promotion and marketing of a treatment method. It is widely appreciated that there is a tendency in the pharmaceutical industry to develop many compounds for well established therapeutic targets. Examples include beta adrenergic blockers, hydroxymethylglutaryl (HMG) CoA reductase inhibitors (statins), dopamine D2 receptor antagonists and serotonin transporter inhibitors. Frequently the pharmacology of these compounds is quite similar in terms of efficacy and side effects. Therefore the marketing of one compound vs. other members of the class is a challenging problem for drug companies, and is reflected in the lesser success that late products typically achieve compared to the first and second approved products. It occurred to the inventors that genetic stratification can provide the basis for identifying a patient population with a superior response rate or improved safety to one member of a class of drugs, and that this information can be the basis for commercialization of that compound. Such a commercialization campaign can be directed at caregivers, particularly physicians, or at patients and their families, or both.

1. Identification of Candidate Genes Relevant to the Action of a Drug

Practice of this invention will often begin with identification of a specific pharmaceutical product, for example a drug, that would benefit from improved efficacy or reduced toxicity or both, and the recognition that pharmacogenetic investigations as described herein provide a basis for achieving such improved characteristics. The question then becomes which genes and variances, such as those provided in this application in Tables 1-6, 12-17, and 18-23, would be most relevant to interpatient variation in response to the drug. As discussed above, the set of relevant genes includes both genes involved in the disease process and genes involved in the interaction of the patient and the treatment – for example genes involved in pharmacokinetic and pharmacodynamic action of a drug. The biological and biomedical literature and online databases provide useful guidance in selecting such genes. Specific guidance in the use of these resources is provided below.

Review the literature and online sources

One way to find genes that affect response to a drug in a particular disease setting is to review the published literature and available online databases regarding the pathophysiology of the disease and the pharmacology of the drug. Literature or
5 online sources can provide specific genes involved in the disease process or drug response, or describe biochemical pathways involving multiple genes, each of which may affect the disease process or drug response.

Alternatively, biochemical or pathological changes characteristic of the disease may be described; such information can be used by one skilled in the art to
10 infer a set of genes that can account for the biochemical or pathologic changes. For example, to understand variation in response to a drug that modulates serotonin levels in a central nervous system (CNS) disorder associated with altered levels of serotonin one would preferably study, at a minimum, variances in genes responsible for serotonin biosynthesis, release from the cell, receptor binding, presynaptic
15 reuptake, and degradation or metabolism. Genes responsible for each of these functions should be examined for variation that may account for interpatient differences in drug response or disease manifestations. As recognized by those skilled in the art, a comprehensive list of such genes can be obtained from textbooks, monographs and the literature.

20 There are several types of scientific information, described in some detail below, that are valuable for identifying a set of candidate genes to be investigated with respect to a specific disease and therapeutic intervention. First there is the medical literature, which provides basic information on disease pathophysiology and therapeutic interventions. A subset of this literature is devoted to specific
25 description of pathologic conditions. Second there is the pharmacology literature, which will provide additional information on the mechanism of action of a drug (pharmacodynamics) as well as its principal routes of metabolic transformation (pharmacokinetics) and the responsible proteins. Third there is the biomedical literature (principally genetics, physiology, biochemistry and molecular biology),
30 which provides more detailed information on metabolic pathways, protein structure and function and gene structure. Fourth, there are a variety of online databases that provide additional information on metabolic pathways, gene families, protein function and other subjects relevant to selecting a set of genes that are likely to affect the response to a treatment.

Medical Literature

A good starting place for information on molecular pathophysiology of a specific disease is a general medical textbook such as Harrison's Principles of Internal Medicine, 14th edition, (2 Vol Set) by A.S. Fauci, E. Braunwald, K.J. Isselbacher, et al. (editors), McGraw Hill, 1997, or Cecil Textbook of Medicine (20th Ed) by R. L. Cecil, F. Plum and J. C. Bennett (Editors) W B Saunders Co., 1996. For pediatric diseases texts such as Nelson Textbook of Pediatrics (15th edition) by R.E. Behrman, R.M. Kliegman, A.M. Arvin and W.E. Nelson (Editors), W B Saunders Co., 1995 or Oski's Principles and Practice of Pediatrics (3rd Edition) by J.A. Mamillan & F.A. Oski Lippincott-Raven, 1999 are useful introductions. For obstetrical and gynecological disorders texts such as Williams Obstetrics (20th Ed) by F.G. Cunningham, N.F. Gant, P.C. McDonald et al. (Editors), Appleton & Lange, 1997 provide general information on disease pathophysiology. For psychiatric disorders texts such as the Comprehensive Textbook of Psychiatry, VI (2 Vols) by H.I. Kaplan and B.J. Sadock (Editors), Lippincott, Williams & Wilkins, 1995, or The American Psychiatric Press Textbook of Psychiatry (3rd edition) by R.E. Hales, S.C. Yudofsky and J.A. Talbott (Editors) Amer Psychiatric Press, 1999 provide an overview of disease nosology, pathophysiological mechanisms and treatment regimens.

In addition to these general texts, there are a variety of more specialized medical texts that provide greater detail about specific disorders which can be utilized in developing a list of candidate genes and variances relevant to interpatient variation in response to a treatment. For example, within the field of medicine there are standard textbooks for each of the subspecialties. Some examples include:

Heart Disease: A Textbook of Cardiovascular Medicine (2 Volume set) by E. Braunwald (Editor), W B Saunders Co., 1996; Hurst's the Heart, Arteries and Veins (9th Ed) (2 Vol Set) by R.W. Alexander, R.C. Schlant, V. Fuster, W. Alexander and E.H. Sonnenblick (Editors) McGraw Hill, 1998; Principles of Neurology (6th edition) by R.D. Adams, M. Victor (editors), and A.H. Ropper (Contributor), McGraw Hill, 1996; Sleisenger & Fordtran's Gastrointestinal and Liver Disease: Pathophysiology, Diagnosis, Management (6th edition) by M. Feldman, B.F. Scharschmidt and M. Sleisenger (Editors), W B Saunders Co., 1997; Textbook of Rheumatology (5th edition) by W.N. Kelley, S. Ruddy, E.D. Harris Jr. and C.B. Sledge (Editors) (2 volume set) W B Saunders Co., 1997; Williams Textbook of Endocrinology (9th edition) by J.D. Wilson, D.W. Foster, H. M. Kronenberg and Larsen (Editors), W B Saunders Co., 1998; Wintrobe's Clinical Hematology (10th Ed) by G.R. Lee, J. Foerster (Editor) and J. Lukens (Editors) (2 Volumes) Lippincott, Williams & Wilkins, 1998; Cancer: Principles & Practice of Oncology

(5th edition) by V.T. Devita, S.A. Rosenberg and S. Hellman (editors), Lippincott-Raven Publishers, 1997; Principles of Pulmonary Medicine (3rd edition) by S.E. Weinberger & J Fletcher (Editors), W B Saunders Co., 1998; Diagnosis and Management of Renal Disease and Hypertension (2nd edition) by A.K. Mandal & J.C. Jennette (Editors), Carolina Academic Press, 1994. Massry & Glassock's Textbook of Nephrology (3rd edition) by S.G. Massry & R.J. Glassock (editors) Williams & Wilkins, 1995; The Management of Pain by J.J. Bonica, Lea and Febiger, 1992; Ophthalmology by M. Yanoff & J.S. Duker, Mosby Year Book, 1998; Clinical Ophthalmology: A Systemic Approach by J.J. Kanski, Butterworth-Heinemann, 1994; and Essential Otolaryngology by J.K. Lee Appleton and Lange 1998.

In addition to these subspecialty texts there are many textbooks and monographs that concern more restricted disease areas, or specific diseases. Such books provide more extensive coverage of pathophysiologic mechanisms and therapeutic options. The number of such books is too great to provide examples for all but a few diseases, however one skilled in the art will be able to readily identify relevant texts. One simple way to search for relevant titles is to use the search engine of an online bookseller such as <http://www.amazon.com> or <http://www.barnesandnoble.com> using the disease or drug (or the group of diseases or drugs to which they belong) as search terms. For example a search for asthma would turn up titles such as Asthma : Basic Mechanisms and Clinical Management (3rd edition) by P.J. Barnes, I.W. Rodger and N.C. Thomson (Editors), Academic Press, 1998 and Airways and Vascular Remodelling in Asthma and Cardiovascular Disease : Implications for Therapeutic Intervention, by C. Page & J. Black (Editors), Academic Press, 1994.

Pathology Literature

In addition to medical texts there are texts that specifically address disease etiology and pathologic changes associated with disease. A good general pathology text is Robbins Pathologic Basis of Disease (6th edition) by R.S. Cotran, V. Kumar, T. Collins and S.L. Robbins, W B Saunders Co., 1998. Specialized pathology texts exist for each organ system and for specific diseases, similar to medical texts. These texts are useful sources of information for one skilled in the art for developing lists of genes that may account for some of the known pathologic changes in disease tissue. Exemplary texts are as follows:

Bone Marrow Pathology 2nd edition, by B.J. Bain, I. Lampert. & D. Clark, Blackwell Science, 1996; Atlas of Renal Pathology by F.G. Silva, W.B. Saunders,

1999; Fundamentals of Toxicologic Pathology by W.M. Haschek and C.G. Rousseaux, Academic Press, 1997; Gastrointestinal Pathology by P. Chandrasoma, Appleton and Lange, 1998; Ophthalmic Pathology with Clinical Correlations by J. Sassani, Lippincott-Raven, 1997; Pathology of Bone and Joint Disorders by F. McCarthy, F.J. Frassica and A. Ross, W. B. Saunders, 1998; Pulmonary Pathology by M.A. Grippi, Lippincott-Raven, 1995; Neuropathology by D. Ellison, L. Chimelli, B. Harding, S. Love & J. Lowe, Mosby Year Book, 1997; Greenfield's Neuropathology 6th edition by J.G. Greenfield, P.L. Lantos & D.I. Graham, Edward Arnold, 1997.

10 *Pharmacology, Pharmacogenetics and Pharmacy Literature*

There are also both general and specialized texts and monographs on pharmacology that provide data on pharmacokinetics and pharmacodynamics of drugs. The discussion of pharmacodynamics (mechanism of action of the drug) in such texts is often supported by a review of the biochemical pathway or pathways that are affected by the drug. Also, proteins related to the target protein are often listed; it is important to account for variation in such proteins as the related proteins may be involved in drug pharmacology. For example, there are 14 known serotonin receptors. Various pharmacological serotonin agonists or antagonists have different affinities for these different receptors. Variation in a specific receptor may affect the pharmacology not only of drugs targeted to that receptor, but also drugs that are principally agonists or antagonists of different receptors. Such compounds may produce different effects on two allelic forms of a non-targeted receptor; for example one variant form may bind the compound with higher affinity than the other, or a compound that is principally an antagonist for one allele may be a partial agonist for another allele. Thus genes encoding proteins structurally related to the target protein should be screened for variance in order to successfully realize the methods of the present invention. A good general pharmacology text is Goodman & Gilman's the Pharmacological Basis of Therapeutics (9th Ed) by J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon and A.G. Gilman (Editors) McGraw Hill, 1996. There are also texts that focus on the pharmacology of drugs for specific disease areas, or specific classes of drugs (e.g. natural products) or adverse drug interactions, among other subjects. Specific examples include:

35 The American Psychiatric Press Textbook of Psychopharmacology (2nd edition) by A.F. Schatzberg & C.B. Nemeroff (Editors), American Psychiatric Press, 1998; and Essential Psychopharmacology : Neuroscientific Basis and Practical Applications by N. Muntner and S.M. Stahl, Cambridge Univ Press, 1996.

There are also texts on pharmacogenetics which are particularly useful for identifying genes which may contribute to variable pharmacokinetic response. In addition there are texts on some of the major xenobiotic metabolizing proteins, such as the cytochrome P450 genes including Pharmacogenetics of Drug Metabolism (International Encyclopedia of Pharmacology and Therapeutics) by Werner Kalow (Editor) Pergamon Press, 1992; Genetic Factors in Drug Therapy : Clinical and Molecular Pharmacogenetics by D.A Price Evans, Cambridge Univ Press, 1993; Pharmacogenetics (Oxford Monographs on Medical Genetics, 32) by W.W. Weber, Oxford Univ Press, 1997; Cytochrome P450 : Structure, Mechanism, and Biochemistry by P.R. Ortiz de Montellano (Editor), Plenum Publishing Corp, 1995; and Appleton & Lange's Review of Pharmacy, 6th edition, (Appleton & Lange's Review Series) by G.D. Hall & B.S. Reiss, Appleton & Lange, 1997.

Genetics, Biochemistry and Molecular Biology Literature

In addition to the medical, pathology, and pharmacology texts listed above there are several information sources that one skilled in the art will turn to for information on the genetic, physiologic, biochemical, and molecular biological aspects of the disease, disorder or condition or the effect of the therapeutic intervention on specific physiologic processes. The biomedical literature may include information on nonhuman organisms that is relevant to understanding the likely disease or pharmacological pathways in man.

Also provided below are illustrative texts which will aid in the identification of a pathway or pathways, and a gene or genes that may be relevant to interindividual variation in response to a therapy. Textbooks of biochemistry, genetics and physiology are often useful sources for such pathway information. In order to ascertain the appropriate methods to analyze the effects of an allelic variance, variances, or haplotypes in vitro, one skilled in the art will review existing information on molecular biology, cell biology, genetics, biochemistry; and physiology. Such texts are useful sources for general and specific information on the genetic and biochemical processes involved in disease and in drug action, as well as experimental procedures that may be useful in performing in vitro research on an allelic variance, variances, or haplotype.

Texts on gene structure and function and RNA biochemistry will be useful in evaluating the consequences of variances that do not change the coding sequence

(silent variances). Such variances may alter the interaction of RNA with proteins or other regulatory molecules affecting RNA processing, polyadenylation, or export.

Molecular and Cellular Biology

Molecular Cell Biology by H. Lodish, D. Baltimore, A. Berk, L. Zipurksy & J. Darnell, W H Freeman & Co., 1995; Essentials of Molecular Biology, D. Freifelder and Malacinski, Jones and Bartlett, 1993; Genes and Genomes: A Changing Perspective, M. Singer and P. Berg, University Science Books, 1991; Gene Structure and Expression, J.D. Hawkins, 1996. Cambridge University Press; Molecular Biology of the Cell, 2nd edition, B. Alberts et al., Garland Publishing, 1994.

Molecular Genetics

The Metabolic and Molecular Bases of Inherited Disease by C. R. Scriver, A.L. Beaudet, W.S. Sly (Editors), 7th edition, McGraw Hill, 1995; Genetics and Molecular Biology, R. Schleif, 1994. 2nd edition, Johns Hopkins University Press; Genetics, P.J. Russell, 1996. 4th edition, Harper Collins; An Introduction to Genetic Analysis, Griffiths et al. 1993. 5th edition, W.H. Freeman and Company; Understanding Genetics: A molecular approach, Rothwell, 1993. Wiley-Liss

General Biochemistry

Biochemistry, L. Stryer, 1995. W.H. Freeman and Company; Biochemistry, D. Voet and J.G. Voet, 1995. John Wiley and Sons; Principles of Biochemistry, A.L. Lehninger, D.L. Nelson, and M.M. Cox, 1993. Worth Publishers; Biochemistry, G. Zubay, 1998. Wm. C. Brown Communications; Biochemistry, C.K. Mathews and K.E. van Holde, 1990. Benjamin/Cummings

Transcription

Eukaryotic Transcription Factors, D.S. Latchman, 1995. Academic Press; Eukaryotic Gene Transcription, S. Goodbourn (ed.), 1996. Oxford University Press; Transcription Factors and DNA Replication, D.S. Pederson and N.H. Heintz, 1994. CRC Press/R.G. Landes Company; Transcriptional Regulation, S.L. McKnight and K. Yamamoto (eds.), 1992. 2 volumes, Cold Spring Harbor Laboratory Press

RNA

Control of Messenger RNA Stability, J. Belasco and G. Brawerman (eds.), 1993. Academic Press; RNA-Protein Interactions, Nagai and Mattaj (eds.), 1994. Oxford

University Press; mRNA Metabolism and Post-transcriptional Gene Regulation, Harford and Morris (eds.), 1997. Wiley-Liss.

Translation

- 5 Translational Control, J.W.B. Hershey, M.B. Mathews, and N. Sonenberg (eds.), 1995. Cold Spring Harbor Laboratory Press

General Physiology

- 10 Textbook of Medical Physiology 9th Edition by A.C. Guyton and J.E. Hall W.B. Saunders, 1997; Review of Medical Physiology, 18th Edition by W.F. Ganong, Appleton and Lange, 1997.

Online Databases

- Those skilled in the art are familiar with how to search the biomedical literature, such as, e.g., libraries, online PubMed, abstract listings, and online
15 mutation databases. One particularly useful resource is maintained at the web site of the National Center for Biotechnology Information (ncbi):
<http://www.ncbi.nlm.nih.gov/>. From the ncbi site one can access Online Mendelian Inheritance in Man (OMIM). OMIM can be found at:
<http://www3.ncbi.nlm.nih.gov/Omim/searchomim.html>. OMIM is a medically
20 oriented database of genetic information with entries for thousands of genes. The OMIM record number is provided for many of the genes in Tables 1-6 and 12-23 (see column 3), and constitutes an excellent entry point for identification of references that point to the broader literature. Another useful site at NCBI is the Entrez browser, located at <http://www3.ncbi.nlm.nih.gov/Entrez/>. One can search
25 genomes, polynucleotides, proteins, 3D structures, taxonomy or the biomedical literature (PubMed) via the Entrez site. More generally links to a number of useful sites with biomedical or genetic data are maintained at sites such as Med Web at the Emory University Health Sciences Center Library:
<http://WWW.MedWeb.Emory.Edu/MedWeb/>; Riken, a Japanese web site at:
30 <http://www.rtc.riken.go.jp/othersite.html> with links to DNA sequence, structural, molecular biology, bioinformatics, and other databases; at the Oak Ridge National Laboratory web site: <http://www.ornl.gov/hgmis/links.html>; or at the Yahoo website of Diseases and Conditions:
http://dir.yahoo.com/health/diseases_and_conditions/index.html. Each of the
35 indicated web sites has additional useful links to other sites.

Another type of database with utility in selecting the genes on a biochemical pathway that may affect the response to a drug are databases that provide information on biochemical pathways. Examples of such databases include the Kyoto Encyclopedia of Genes and Genomes (KEGG), which can be found at:
5 <http://www.genome.ad.jp/kegg/kegg.html>. This site has pictures of many biochemical pathways, as well as links to other metabolic databases such as the well known Boehringer Mannheim biochemical pathways charts:
<http://www.expasy.ch/cgi-bin/search-biochem-index>. The metabolic charts at the
10 latter site are comprehensive, and excellent starting points for working out the salient enzymes on any given pathway.

Each of the web sites mentioned above has links to other useful web sites, which in turn can lead to additional sites with useful information. *Research Libraries*

Those skilled in the art will often require information found only at large libraries. The National Library of Medicine (<http://www.nlm.nih.gov/>) is the largest
15 medical library in the world and its catalogs can be searched online. Other libraries, such as university or medical school libraries are also useful to conduct searches. Biomedical books such as those referred to above can often be obtained from online bookstores as described above.

Biomedical Literature

20 To obtain up to date information on drugs and their mechanism of action and biotransformation; disease pathophysiology; biochemical pathways relevant to drug action and disease pathophysiology; and genes that encode proteins relevant to drug action and disease one skilled in the art will consult the biomedical literature. A widely used, publically accessible web site for searching published journal articles is
25 PubMed (<http://www.ncbi.nlm.nih.gov/PubMed/>). At this site, one can search for the most recent articles (within the last 1-2 months) or older literature (back to 1966). Many Journals also have their own sites on the world wide web and can be searched online. For example see the IDEAL web site at:
<http://www.apnet.com/www/ap/aboutid.html>. This site is an online library,
30 featuring full text journals from Academic Press and selected journals from W.B. Saunders and Churchill Livingstone. The site provides access (for a fee) to nearly 2000 scientific, technical, and medical journals.

Experimental methods for identification of genes involved in the action of a drug

There are a number of experimental methods for identifying genes and gene products that mediate or modulate the effects of a drug or other treatment. They encompass analyses of RNA and protein expression as well as methods for detecting protein – protein interactions and protein – ligand interactions. Two preferred experimental methods for identification of genes that may be involved in the action of a drug are (1) methods for measuring the expression levels of many mRNA transcripts in cells or organisms treated with the drug (2) methods for measuring the expression levels of many proteins in cells or organisms treated with the drug.

RNA transcripts or proteins that are substantially increased or decreased in drug treated cells or tissues relative to control cells or tissues are candidates for mediating the action of the drug. Preferably the level of an mRNA is at least 30% higher or lower in drug treated cells, more preferably at least 50% higher or lower, and most preferably two fold higher or lower than levels in non-drug treated control cells. The analysis of RNA levels can be performed on total RNA or on polyadenylated RNA selected by oligodT affinity. Further, RNA from different cell compartments can be analyzed independently – for example nuclear vs. cytoplasmic RNA. In addition to RNA levels, RNA kinetics can be examined, or the pool of RNAs currently being translated can be analyzed by isolation of RNA from polysomes. Other useful experimental methods include protein interaction methods such as the yeast two hybrid system and variants thereof which facilitate the detection of protein – protein interactions. Preferably one of the interacting proteins is the drug target or another protein strongly implicated in the action of the compound being assessed.

The pool of RNAs expressed in a cell is sometimes referred to as the transcriptome. Methods for measuring the transcriptome, or some part of it, are known in the art. A recent collection of articles summarizing some current methods appeared as a supplement to the journal *Nature Genetics*. (The Chipping Forecast. *Nature Genetics* supplement, volume 21, January 1999.) A preferred method for measuring expression levels of mRNAs is to spot PCR products corresponding to a large number of specific genes on a nylon membrane such as Hybond N Plus (Amersham-Pharmacia). Total cellular mRNA is then isolated, labelled by random oligonucleotide priming in the presence of a detectable label (e.g. alpha 33P labelled radionucleotides or dye labelled nucleotides), and hybridized with the filter containing the PCR products. The resulting signals can be analyzed by commercially available software, such as can be obtained from Clontech/Molecular Dynamics or Research Genetics, Inc.

Experiments have been described in model systems that demonstrate the utility of measuring changes in the transcriptome before and after changing the growth conditions of cells, for example by changing the nutrient environment. The changes in gene expression help reveal the network of genes that mediate physiological responses to the altered growth condition. Similarly, the addition of a drug to the cellular or in vivo environment, followed by monitoring the changes in gene expression can aid in identification of gene networks that mediate pharmacological responses.

The pool of proteins expressed in a cell is sometimes referred to as the proteome. Studies of the proteome may include not only protein abundance but also protein subcellular localization and protein-protein interaction. Methods for measuring the proteome, or some part of it, are known in the art. One widely used method is to extract total cellular protein and separate it in two dimensions, for example first by size and then by isoelectric point. The resulting protein spots can be stained and quantitated, and individual spots can be excised and analyzed by mass spectrometry to provide definitive identification. The results can be compared from two or more cell lines or tissues, at least one of which has been treated with a drug. The differential up or down modulation of specific proteins in response to drug treatment may indicate their role in mediating the pharmacologic actions of the drug. Another way to identify the network of proteins that mediate the actions of a drug is to exploit methods for identifying interacting proteins. By starting with a protein known to be involved in the action of a drug – for example the drug target – one can use systems such as the yeast two hybrid system and variants thereof (known to those skilled in the art; see Ausubel et al., *Current Protocols in Molecular Biology*, op. cit.) to identify additional proteins in the network of proteins that mediate drug action. The genes encoding such proteins would be useful for screening for DNA sequence variances, which in turn may be useful for analysis of interpatient variation in response to treatments. For example, the protein 5-lipoxygenase (5LO) is an enzyme which is at the beginning of the leukotriene biosynthetic pathway and is a target for anti-inflammatory drugs used to treat asthma and other diseases. In order to detect proteins that interact with 5-lipoxygenase the two-hybrid system was recently used to isolate three different proteins, none previously known to interact with 5LO. (Provost et al., *Interaction of 5-lipoxygenase with cellular proteins. Proc. Natl. Acad. Sci. U.S.A.* 96: 1881-1885, 1999.) A recent collection of articles summarizing some current methods in proteomics appeared in the August 1998 issue of the journal *Electrophoresis* (volume 19, number 11). Other useful articles include: Blackstock WP, et al.

Proteomics: quantitative and physical mapping of cellular proteins. *Trends Biotechnol.* 17 (3): p. 121-7, 1999, and Patton W.F., Proteome analysis II. Protein subcellular redistribution: linking physiology to genomics via the proteome and separation technologies involved. *J Chromatogr B Biomed Sci App.* 722(1-2):203-23. 1999.

Since many of these methods can also be used to assess whether specific polymorphisms are likely to have biological effects, they are also relevant in section 3, below, concerning methods for assessing the likely contribution of variances in candidate genes to clinical variation in patient responses to therapy.

2. Screen for Variances in Genes that may be Related to Therapeutic Response

Having identified a set of genes that may affect response to a drug the next step is to screen the genes for variances that may account for interindividual variation in response to the drug. There are a variety of levels at which a gene can be screened for variances, and a variety of methods for variance screening. The two main levels of variance screening are genomic DNA screening and cDNA screening. Genomic variance detection may include screening the entire genomic segment spanning the gene from 2 kb to 10 kb upstream of the transcription start site to the polyadenylation site, or 2 to 10 kb beyond the polyadenylation site. Alternatively genomic variance detection may (for intron containing genes) include the exons and some region around them containing the splicing signals, for example, but not all of the intronic sequences. In addition to screening introns and exons for variances it is generally desirable to screen regulatory DNA sequences for variances. Promoter, enhancer, silencer and other regulatory elements have been described in human genes. The promoter is generally proximal to the transcription start site, although there may be several promoters and several transcription start sites. Enhancer, silencer and other regulatory elements may be intragenic or may lie outside the introns and exons, possibly at a considerable distance, such as 100 kb away. Variances in such sequences may affect basal gene expression or regulation of gene expression. In either case such variation may affect the response of an individual patient to a therapeutic intervention, for example a drug, as described in the examples. Thus in practicing the present invention it is useful to screen regulatory sequences as well as transcribed sequences, in order to identify variances that may affect gene transcription. Frequently the genomic sequence of a gene can be found in the sources above, particularly by searching GenBank or Medline (PubMed). The name of the gene can be entered at a site such as Entrez: <http://www.ncbi.nlm.nih.gov/Entrez/nucleotide.html>. Using the genomic sequence

and information from the biomedical literature one skilled in the art can perform a variance detection procedure such as those described in examples 2, 3, 4.

Variance detection is often first performed on the cDNA of a gene for several reasons. First, available data on functional sequence variances suggests that
5 variances in the transcribed portion of a gene may be most likely to have functional consequences as they can affect the interaction of the transcript with a wide variety of cellular factors during the complex processes of RNA transcription, processing and translation, with consequent effects on RNA splicing, stability, translational efficiency or other processes. Second, as a practical matter the cDNA sequence of a
10 gene is often available before the genomic structure is known, although the reverse will be true in the future as the sequence of the human genome is determined. Third, the cDNA is often compact compared to the genomic locus, and can be screened for variances with much less effort. If the genomic structure is not known then only the cDNA sequence can be scanned for variances. Methods for preparing cDNA are
15 described in Example 1. Methods for variance detection on cDNA are described below and in the examples.

In general it is preferable to catalog genetic variation at the genomic DNA level because there are an increasing number of well documented instances of functionally important variances that lie outside of transcribed sequence. Also, to
20 properly use optimal genetic methods to assess the contribution of a candidate gene to variation in a phenotype of interest it is desirable to understand the character of sequence variation in the candidate gene: what is the nature of linkage disequilibrium between different variances in the gene; are there sites of recombination within the gene; what is the extent of homoplasy in the gene (i.e.
25 occurrence of two variant sites that are identical by state but not identical by descent because the same variance arose at least twice in human evolutionary history on two different haplotypes); what are the different haplotypes and how can they be grouped to increase the power of genetic analysis?

Methods for variance screening have been described, including DNA
30 sequencing. See for example: US5698400: Detection of mutation by resolvase cleavage; US5217863: Detection of mutations in nucleic acids; and US5750335: Screening for genetic variation, as well as the examples and references cited therein for examples of useful variance detection procedures. Detailed variance detection procedures are also described in examples 2, 3, 4. One skilled in the art will
35 recognize that depending on the specific aims of a variance detection project (number of genes being screened, number of individuals being screened, total length

of DNA being screened) one of the above cited methods may be preferable to the others, or yet another procedure may be optimal. A preferred method of variance detection is chain terminating DNA sequencing using dye labeled primers, cycle sequencing and software for assessing the quality of the DNA sequence as well as specialized software for calling heterozygotes. The use of such procedures has been described by Nickerson and colleagues. See for example: Rieder M.J., et al. Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome. *Nucleic Acids Res.* 26 (4):967-73, 1998, and: Nickerson D.A., et al. PolyPhred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. *Nucleic Acids Res.* 25 (14):2745-51, 1997. Although the variances provided in Tables 12-17, and 18-23 consist principally of cDNA variances, it is an aspect of this invention that detection of genomic variances is also a useful method for identification of variances that may account for interpatient variation in response to a therapy.

Another important aspect of variance detection is the use of DNA from a panel of human subjects that represents a known population. For example, if the subjects are being screened for variances relevant to a specific drug development program it is desirable to include both subjects with the target disease and healthy subjects in the panel, because certain variances may occur at different frequencies in the healthy and disease populations and can only be reliably detected by screening both populations. Also, for example, if the drug development program is taking place in Japan, it is important to include Japanese individuals in the screening population. In general, it is always desirable to include subjects of known geographic, racial or ethnic identity in a variance screening experiment so the results can be interpreted appropriately for different patient populations, if necessary. Also, in order to select optimal sets of variances for genetic analysis of a gene locus it is desirable to know which variances have occurred recently – perhaps on multiple different chromosomes - and which are ancient. Inclusion of one or more apes or monkeys in the variance screening panel is one way of gaining insight into the evolutionary history of variances. Chimpanzees are preferred subjects for inclusion in a variance screening panel.

3. Assess the Likely Contribution of Variances in Candidate Genes to Clinical Variation in Patient Responses to Therapy

Once a set of genes likely to affect disease pathophysiology or drug action has been identified, and those genes have been screened for variances, said variances

(e.g., provided in Tables 12-17, and 18-23) can be assessed for their contribution to variation in the pharmacological or toxicological phenotypes of interest. Such studies are useful for reducing a large number of candidate variances to a smaller number of variances to be tested in clinical trials. There are several methods which
5 can be used in the present invention for assessing the medical and pharmaceutical implications of a DNA sequence variance. They range from computational methods to *in vitro* and/or *in vivo* experimental methods, to prospective human clinical trials, and also include a variety of other laboratory and clinical measures that can provide evidence of the medical consequences of a variance. In general, human clinical
10 trials constitute the highest standard of proof that a variance or set of variances is useful for selecting a method of treatment, however, computational and *in vitro* data, or retrospective analysis of human clinical data may provide strong evidence that a particular variance will affect response to a given therapy, often at lower cost and in less time than a prospective clinical trial. Moreover, at an early stage in the analysis
15 when there are many possible hypotheses to explain interpatient variation in treatment response, the use of informatics-based approaches to evaluate the likely functional effects of specific variances is an efficient way to proceed.

Informatics-based approaches to the prediction of the likely functional effects of variances include DNA and protein sequence analysis (phylogenetic
20 approaches and motif searching) and protein modeling (based on coordinates in the protein database, or pdb; see <http://www.rcsb.org/pdb/>). See, for example: Kawabata et al. The Protein Mutant Database. *Nucleic Acids Research* 27: 355-357, 1999; also available at: <http://pmd.ddbj.nig.ac.jp>. Such analyses can be performed quickly and inexpensively, and the results may allow selection of certain genes for
25 more extensive *in vitro* or *in vivo* studies or for more variance detection or both.

The three dimensional structure of many medically and pharmaceutically important proteins, or homologs of such proteins in other species, or examples of domains present in such proteins, is known as a result of x-ray crystallography studies and, increasingly, nuclear magnetic resonance studies. Further, there are
30 increasingly powerful tools for modeling the structure of proteins with unsolved structure, particularly if there is a related (homologous) protein with known structure. (For reviews see: Rost et al., Protein fold recognition by prediction-based threading, *J. Mol. Biol.* 270:471-480, 1997; Firestine et al., Threading your way to protein function, *Chem. Biol.* 3:779-783, 1996) There are also powerful methods for
35 identifying conserved domains and vital amino acid residues of proteins of unknown structure by analysis of phylogenetic relationships. (Deleage et al., Protein structure prediction: Implications for the biologist, *Biochimie* 79:681-686, 1997; Taylor et al., Multiple protein structure alignment, *Protein Sci.* 3:1858-1870, 1994) These

methods can permit the prediction of functionally important variances, either on the basis of structure or evolutionary conservation. For example, a crystal structure can reveal which amino acids comprise a small molecule binding site. The identification of a polymorphic amino acid variance in the topological neighborhood of such a site, and, in particular, the demonstration that at least one variant form of the protein has a variant amino acid which impinges on (or which may otherwise affect the chemical environment around) the small molecule binding pocket differently from another variant form, provides strong evidence that the variance may affect the function of the protein. From this it follows that the interaction of the protein with a treatment method, such an administered compound, will likely be variable between different patients. One skilled in the art will recognize that the application of computational tools to the identification of functionally consequential variances involves applying the knowledge and tools of medicinal chemistry and physiology to the analysis.

Phylogenetic approaches to understanding sequence variation are also useful. Thus if a sequence variance occurs at a nucleotide or encoded amino acid residue where there is usually little or no variation in homologs of the protein of interest from non-human species, particularly evolutionarily remote species, then the variance is more likely to affect function of the RNA or protein. Computational methods for phylogenetic analysis are known in the art, (see below for citations of some methods).

Computational methods are also useful for analyzing DNA polymorphisms in transcriptional regulatory sequences, including promoters and enhancers. One useful approach is to compare variances in potential or proven transcriptional regulatory sequences to a catalog of all known transcriptional regulatory sequences, including consensus binding domains for all transcription factor binding domains. See, for example, the databases cited in: Burks, C. Molecular Biology Database List. *Nucleic Acids Research* 27: 1-9, 1999, and links to useful databases on the internet at:

http://www.oup.co.uk/nar/Volume_27/issue_01/summary/gkc105_gml.html. In particular see the Transcription Factor Database (Heinemeyer, T., et al. (1999) Expanding the TRANSFAC database towards an expert system of regulatory molecular mechanisms. *Nucleic Acids Res.* 27: 318-322, or on the internet at: <http://193.175.244.40/TRANSFAC/index.html>). Any sequence variances in transcriptional regulatory sequences can be assessed for their effects on mRNA levels using standard methods, either by making plasmid constructs with the different allelic forms of the sequence, transfecting them into cells and measuring the output of a reporter transcript, or by assays of cells with different endogenous

alleles of variances. One example of a polymorphism in a transcriptional regulatory element that has a pharmacogenetic effect is described by Drazen et al. (1999) Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nature Genetics* 22: 168-170. Drazen and co-workers
5 found that a polymorphism in an Sp1-transcription factor binding domain, which varied among subjects from 3-6 tandem copies, accounted for varied expression levels of the 5-lipoxygenase gene when assayed in vitro in reporter construct assays. This effect would have been flagged by an informatics analysis that surveyed the 5-lipoxygenase candidate promoter region for transcriptional regulatory sequences
10 (resulting in discovery of polymorphism in the Sp1 motif).

4. Perform *in vitro* or *in vivo* Experiments to Assess the Functional Importance of Gene Variances

There are two broad types of studies useful for assessing the likely
15 importance of variances: analysis of RNA or protein abundance (as described above in the context of methods for identifying candidate genes for explaining interpatient variation in treatment response) or analysis of functional differences in different variant forms of a gene, mRNA or protein. Studies of functional differences may involve direct measurements of biochemical activity of different variant forms of an
20 mRNA or protein, or may involve assaying the influence of a variance or variances on various cell properties, including both tissue culture and *in vivo* studies.

The selection of an appropriate experimental program for testing the medical consequences of a variance may differ depending on the nature of the variance, the gene, and the disease. For example if there is already evidence that a protein is
25 involved in the pharmacologic action of a drug, then the *in vitro* or *in vivo* demonstration that an amino acid variance in the protein affects its biochemical activity is strong evidence that the variance will have an effect on the pharmacology of the drug in patients, and therefore that patients with different variant forms of the gene may have different responses to the same dose of drug. If the variance is silent
30 with respect to protein coding information, or if it lies in a noncoding portion of the gene (e.g., a promoter, an intron, or a 5'- or 3'-untranslated region) then the appropriate biochemical assay may be to assess mRNA abundance, half life, or translational efficiency. If, on the other hand, there is no substantial evidence that the protein encoded by a particular gene is relevant to drug pharmacology, but
35 instead is a candidate gene on account of its involvement in disease pathophysiology, then the optimal test may be a clinical study addressing whether two patient groups distinguished on the basis of the variance respond differently to a therapeutic intervention. This approach reflects the current reality that biologists do

not sufficiently understand gene regulation, gene expression and gene function to consistently make accurate inferences about the consequences of DNA sequence variances for pharmacological responses.

In summary, if there is a plausible hypothesis regarding the effect of a protein on the action of a drug, then *in vitro* and *in vivo* approaches, including those described below, will be useful to predict whether a given variance is therapeutically consequential. If, on the other hand, there is no evidence of such an effect, then the preferred test is an empirical clinical measure of the impact to the variance on efficacy or toxicity *in vivo* (which requires no evidence or assumptions regarding the mechanism by which the variance may exert an effect on a therapeutic response). However, given the expense and statistical constraints of clinical trials, it is preferable to limit clinical testing to variances for which there is at least some experimental or computational evidence of a functional effect.

Experimental Methods: Genomic DNA Analysis

Variances in DNA may affect the basal transcription or regulated transcription of a gene locus. Such variances may be located in any part of the gene but are most likely to be located in the promoter region, the first intron, or in 5' or 3' flanking DNA, where enhancer or silencer elements may be located. Methods for analyzing transcription are well known to those skilled in the art and exemplary methods are briefly described above and in some of the texts cited elsewhere in this application. Transcriptional run off assay is one useful method. Detailed protocols can be found in texts such as: Current Protocols in Molecular Biology edited by: F.M. Ausubel, et al. John Wiley & Sons, Inc, 1999, or: Molecular Cloning: A Laboratory Manual by J. Sambrook, E.F. Fritsch and T Maniatis. 1989. 3 vols, 2nd edition, Cold Spring Harbor Laboratory Press

Experimental Methods: RNA Analysis

RNA variances may affect a wide range of processes including RNA splicing, polyadenylation, capping, export from the nucleus, interaction with translation initiation, elongation or termination factors, or the ribosome, or interaction with cellular factors including regulatory proteins, or factors that may affect mRNA half life. However, the effect of most RNA sequence variances on RNA function, if any, should ultimately be measurable as an effect on RNA or protein levels – either basal levels or regulated levels or levels in some abnormal cell state, such as cells from patients with a disease. Therefore, one preferred method for assessing the effect of RNA variances on RNA function is to measure the levels of RNA produced by different alleles in one or more conditions of cell or tissue

growth. Said measuring can be done by conventional methods such as Northern blots or RNAase protection assays (kits available from Ambion, Inc.), or by methods such as the Taqman assay (developed by the Applied Biosystems Division of the Perkin Elmer Corporation), or by using arrays of oligonucleotides or arrays of cDNAs attached to solid surfaces. Systems for arraying cDNAs are available commercially from companies such as Nanogen and General Scanning. Complete systems for gene expression analysis are available from companies such as Molecular Dynamics. For recent reviews of systems for high throughput RNA expression analysis see the supplement to volume 21 of Nature Genetics entitled "The Chipping Forecast", especially articles beginning on pages 9, 15, 20 and 25.

Additional methods for analyzing the effect of variances on RNA include secondary structure probing, and direct measurement of half life or turnover. Secondary structure can be determined by techniques such as enzymatic probing (using enzymes such as T1, T2 and S1 nuclease), chemical probing or RNAase H probing using oligonucleotides. Most RNA structural assays are performed *in vitro*, however some techniques can be performed on cell extracts or even in living cells, using fluorescence resonance energy transfer to monitor the state of RNA probe molecules.

Experimental Methods: Protein Analysis

There are a variety of experimental methods for investigating the effect of an amino acid variance on response of a patient to a treatment. The preferred method will depend on the availability of cells expressing a particular protein, and the feasibility of a cell-based assay vs. assays on cell extracts, on proteins produced in a foreign host, or on proteins prepared by *in vitro* translation.

For example, the methods and systems listed below can be utilized to demonstrate differential expression, stability and/or activity of different variant forms of a protein, or in phenotype/genotype correlations in a model system.

For the determination of protein levels or protein activity a variety of techniques are available. The *in vitro* protein activity can be determined by transcription or translation in bacteria, yeast, baculovirus, COS cells (transient), Chinese Hamster Ovary (CHO) cells, or studied directly in human cells, or other cell systems can be used. Further, one can perform pulse chase experiments to determine if there are changes in protein stability (half-life).

One skilled in the art can construct cell based assays of protein function, and then perform the assays in cells with different genotypes or haplotypes. For example, identification of cells with different genotypes, e.g. cell lines established from families and subsequent determination of relevant protein phenotypes (e.g.

expression levels, post translational modifications, activity assays) may be performed using standard methods.

Assays of protein levels or function can also be performed on cell lines (or extracts from cell lines) derived from pedigrees in order to determine whether there is a genetic component to variation in protein levels or function. The experimental analysis is as above for RNAs, except the assays are different. Experiments can be performed on naive cells or on cells subjected to various treatments, including pharmacological treatments.

In another approach to the study of amino acid variances one can express genes corresponding to different alleles in experimental organisms and examine effects on disease phenotype (if relevant in the animal model), or on response to the presence of a compound. Such experiments may be performed in animals that have disrupted copies of the homologous gene (e.g. gene knockout animals engineered to be deficient in a target gene); or variant forms of the human gene may be introduced into germ cells by transgenic methods, or a combination of approaches may be used. To create animal strains with targeted gene disruptions a DNA construct is created (using DNA sequence information from the host animal) that will undergo homologous recombination when inserted into the nucleus of an embryonic stem cell. The targeted gene is effectively inactivated due to the insertion of non-natural sequence – for example a translation stop codon or a marker gene sequence that interrupts the reading frame. Well known PCR based methods are then used to screen for those cells in which the desired homologous recombination event has occurred. Gene knockouts can be accomplished in worms, drosophila, mice or other organisms. Once the knockout cells are created (in whatever species) the candidate therapeutic intervention can be administered to the animal and pharmacological or biological responses measured, including gene expression levels. If variant forms of the gene are useful in explaining interpatient variation in response to the compound in man, then complete absence of the gene in an experimental organism should have a major effect on drug response. As a next step various human forms of the gene can be introduced into the knockout organism (a technique sometimes referred to as a knock-in). Again, pharmacological studies can be performed to assess the impact of different human variances on drug response. Methods relevant to the experimental approaches described above can be found in the following exemplary texts:

35 *General Molecular Biology Methods*

Molecular Biology: A project approach, S.J. Karcher, Fall 1995. Academic Press; DNA Cloning: A Practical Approach, D.M. Glover and B.D. Hayes (eds). 1995. IRL/Oxford University Press. Vol. 1 - Core Techniques; Vol 2 - Expression Systems; Vol. 3 -

Complex Genomes; Vol. 4 -Mammalian Systems; Short Protocols in Molecular Biology, Ausubel et al. October 1995. 3rd edition, John Wiley and Sons; Current Protocols in Molecular Biology Edited by: F.M. Ausubel, R.Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, K. Struhl, (Series Editor: V.B. Chanda), 1988; Molecular Cloning: A laboratory manual, J. Sambrook, E.F. Fritsch. 1989. 3 vols, 2nd edition, Cold Spring Harbor Laboratory Press.

Polymerase chain reaction (PCR)

- PCR Primer: A laboratory manual, C.W. Diffenbach and G.S. Dveksler (eds.). 1995. Cold Spring Harbor Laboratory Press; The Polymerase Chain Reaction, K.B. Mullis et al. (eds.), 1994. Birkhauser; PCR Strategies, M.A. Innis, D.H. Gelf, and J.J. Sninsky (eds.), 1995. Academic Press.

General procedures for discipline specific studies

- Current Protocols in Neuroscience Edited by: J. Crawley, C. Gerfen, R. McKay, M. Rogawski, D. Sibley, P. Skolnick, (Series Editor: G. Taylor), 1997; Current Protocols in Pharmacology Edited by: S. J. Enna / M. Williams, J.W. Ferkany, T. Kenakin, R.E. Porsolt, J.P. Sullivan, (Series Editor: G. Taylor), 1998; Current Protocols in Protein Science Edited by: J.E. Coligan, B.M. Dunn, H.L. Ploegh, D.W. Speicher, P.T. Wingfield, (Series Editor: Virginia Benson Chanda), 1995; Current Protocols in Cell Biology Edited by: J.S. Bonifacino, M. Dasso, J. Lippincott-Schwartz, J.B. Harford, K.M. Yamada, (Series Editor: K. Morgan) 1999; Current Protocols in Cytometry Managing Editor: J.P. Robinson, Z. Darzynkiewicz (ed) / P. Dean (ed), A. Orfao (ed), P. Rabinovitch (ed), C. Stewart (ed), H. Tanke (ed), L. Wheelless (ed), (Series Editor: J. Paul Robinson), 1997; Current Protocols in Human Genetics Edited by: N.C. Dracopoli, J.L. Haines, B.R. Korf, et al., (Series Editor: A. Boyle), 1994; Current Protocols in Immunology Edited by: J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, (Series Editor: R. Coico), 1991.

IV. Clinical Trials

- A clinical trial is the definitive test of the utility of a variance or variances for the selection of optimal therapy. A clinical trial in which an interaction of gene variances and clinical outcomes (desired or undesired) is explored will be referred to herein as a "pharmacogenetic clinical trial". Pharmacogenetic clinical trials require no knowledge of the biological function of the gene containing the variance or variances to be assessed, nor any knowledge of how the therapeutic intervention to be assessed works at a biochemical level. The pharmacogenetics effects of a variance can be addressed at a purely statistical level: either a particular variance or

set of variances is consistently associated with a significant difference in a salient drug response parameter (e.g. response rate, effective dose, side effect rate, etc.) or not. On the other hand, if there is information about either the biochemical basis of a therapeutic intervention or the biochemical effects of a variance, then a
5 pharmacogenetic clinical trial can be designed to test a specific hypothesis. In preferred embodiments of the methods of this application the mechanism of action of the compound to be genetically analyzed is at least partially understood.

Methods for performing clinical trials are well known in the art. (see e.g. Guide to Clinical Trials by Bert Spilker, Raven Press, 1991; The Randomized
10 Clinical Trial and Therapeutic Decisions by Niels Tygstrup (Editor), Marcel Dekker; Recent Advances in Clinical Trial Design and Analysis (Cancer Treatment and Research, Ctar 75) by Peter F. Thall (Editor) Kluwer Academic Pub, 1995. Clinical Trials: A Methodologic Perspective by Steven Piantadosi, Wiley Series in Probability and Statistics, 1997). However, performing a clinical trial to test the
15 genetic contribution to interpatient variation in drug response entails additional design considerations, including (i) defining the genetic hypothesis or hypotheses, (ii) devising an analytical strategy for testing the hypothesis, including determination of how many patients will need to be enrolled to have adequate statistical power to measure an effect of a specified magnitude (power analysis), (iii)
20 definition of any primary or secondary genetic endpoints, and (iv) definition of methods of statistical genetic analysis, as well as other aspects. In the outline below some of the major types of genetic hypothesis testing, power analysis and statistical testing and their application in different stages of the drug development process are reviewed. One skilled in the art will recognize that certain of the methods will be
25 best suited to specific clinical situations, and that additional methods are known and can be used in particular instances.

A. Performing a Clinical Trial: Overview

As used herein, a "clinical trial" is the testing of a therapeutic intervention in
30 a volunteer human population for the purpose of determining whether it is safe and/or efficacious in the treatment of a disease, disorder, or condition. The present invention describes methods for achieving superior efficacy and/or safety in a genetically defined subgroup defined by the presence or absence of at least one gene sequence variance, compared to the effect that could be obtained in a conventional
35 trial (without genetic stratification).

A "clinical study" is that part of a clinical trial that involves determination of the effect of a candidate therapeutic intervention on human subjects. It includes clinical evaluation of physiologic responses including pharmacokinetic (bioavailability as affected by drug absorption, distribution, metabolism and excretion) and pharmacodynamic (physiologic response and efficacy) parameters. A pharmacogenetic clinical study (or clinical trial) is a clinical study that involves testing of one or more specific hypotheses regarding the interaction of a genetic variance or variances (or set of variances, i.e. haplotype or haplotypes) on response to a therapeutic intervention. Pharmacogenetic hypotheses are formulated before the study, and may be articulated in the study protocol in the form of primary or secondary endpoints. For example an endpoint may be that in a particular genetic subgroup the rate of objectively defined responses exceeds the response rate in a control group (either the entire control group or the subgroup of controls with the same genetic signature as the treatment subgroup they are being compared to) or exceeds that in the whole treatment group (i.e. without genetic stratification) by some predefined relative or absolute amount.

For a clinical study to commence enrollment and proceed to treat subjects at an institution that receives any federal support (most medical institutions in the US), an application that describes in detail the scientific premise for the therapeutic intervention and the procedures involved in the study, including the endpoints and analytical methods to be used in evaluating the data, must be reviewed and accepted by a review panel, often termed an Institutional Review Board (IRB). Similarly any clinical study that will ultimately be evaluated by the FDA as part of a new drug or product application (or other application as described below), must be reviewed and approved by an IRB. The IRB is responsible for determining that the trial protocol is safe, conforms to established ethical principles and guidelines, has risks proportional to any expected benefits, assures equitable selection of patients, provides sufficient information to patients (via a consent form) to insure that they can make an informed decision about participation, and insures the privacy of participants and the confidentiality of any data collected. (See the report of the National Commission for Protection of Human Subjects of Biomedical and Behavioral Research (1978). The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research. Washington, D.C.: DHEW Publication Number (OS) 78-0012. For a recent review see: Coughlin, S.S. (ed.) (1995) Ethics in Epidemiology and Clinical Research. Epidemiology Resources, Newton, MA.) The European counterpart of the US FDA is the European Medicines Evaluation Agency (EMA). Similar agencies exist in other

countries and are responsible for insuring, via the regulatory process, that clinical trials conform to similar standards as are required in the US. The documents reviewed by an IRB include a clinical protocol containing the information described above, and a consent form.

5 It is also customary, but not required, to prepare an investigator's brochure which describes the scientific hypothesis for the proposed therapeutic intervention, the preclinical data, and the clinical protocol. The brochure is made available to any physician participating in the proposed or ongoing trial.

10 The supporting preclinical data is a report of all the *in vitro*, *in vivo* animal or previous human trial or other data that supports the safety and/or efficacy of a given therapeutic intervention. In a pharmacogenetic clinical trial the preclinical data may also include a description of the effect of a specific genetic variance or variances on biochemical or physiologic experimental variables *in vitro* or *in vivo*, or on treatment outcomes, as determined by *in vivo* studies in animals or humans (for example in an
15 earlier trial), or by retrospective genetic analysis of clinical trial or other medical data (see below) used to formulate or strengthen a pharmacogenetic hypothesis. For example, case reports of unusual pharmacological responses in individuals with rare alleles (e.g. mutant alleles), or the observation of clustering of pharmacological responses in family members may provide the rationale for a pharmacogenetic
20 clinical trial.

The clinical protocol provides the relevant scientific and therapeutic introductory information, describes the inclusion and exclusion criteria for human subject enrollment, including genetic criteria if relevant (e.g. if genotype is to be among the enrollment criteria), describes in detail the exact procedure or procedures
25 for treatment using the candidate therapeutic intervention, describes laboratory analyses to be performed during the study period, and further describes the risks (both known and possible) involving the use of the experimental candidate therapeutic intervention. In a clinical protocol for a pharmacogenetic clinical trial, the clinical protocol will further describe the genetic variance and/or variances
30 hypothesized to account for differential responses in the normal human subjects or patients and supporting preclinical data, if any, a description of the methods for genotyping, genetic data collection and data handling as well as a description of the genetic statistical analysis to be performed to measure the interaction of the variance or variances with treatment response. Further, the clinical protocol for a
35 pharmacogenetic clinical trial will include a description of the genetic study design. For example patients may be stratified by genotype and the response rates in the different groups compared, or patients may be segregated by response and the genotype frequencies in the different responder or nonresponder groups measured.

One or more gene sequence variances or a combination of variances and/or haplotypes may be studied.

The informed consent document is a description of the therapeutic intervention and the clinical protocol in simple language (e.g. third grade level) for the patient to read, understand, and, if willing, agree to participate in the study by signing the document. In a pharmacogenetic clinical study the informed consent document will describe, in simple language, the use of a genetic test or a limited set of genetic tests to determine the subject or patient's genotype at a particular gene variance or variances, and to further ascertain whether, in the study population, particular variances are associated with particular clinical or physiological responses. The consent form should also describe procedures for assuring privacy and confidentiality of genetic information.

The US FDA reviews proposed clinical trials through the process of an Investigational New Drug Application (IND). The IND is composed of the investigator's brochure, the supporting *in vitro* and *in vivo* animal or previous human data, the clinical protocol, and the informed consent forms. In each of the sections of the IND, a specific description of a single allelic variance or a number of variances to be tested in the clinical study will be included. For example, in the investigator's brochure a description of the gene or genes hypothesized to account, at least in part, for differential responses will be included as well as a description of a genetic variance or variances in one or more candidate genes. Further, the preclinical data may include a description of *in vivo*, *in vitro* or *in silico* studies of the biochemical or physiologic effects of a variance or variances (e.g., haplotype) in a candidate gene or genes, as well as the predicted effects of the variance or variances on efficacy or toxicology of the candidate therapeutic intervention. The results of retrospective genetic analysis of response data in patients treated with the candidate therapy may be the basis for formulating the genetic hypotheses to be tested in the prospective trial. The US FDA reviews applications with particular attention to safety and toxicological data to ascertain whether candidate compounds should be tested in humans.

The established phases of clinical development are Phase I, II, III, and IV. The fundamental objectives for each phase become increasingly complex as the stages of clinical development progress. In Phase I, safety in humans is the primary focus. In these studies, dose-ranging designs establish whether the candidate therapeutic intervention is safe in the suspected therapeutic concentration range. However, it is common practice to obtain information about surrogate markers of efficacy even in phase I clinical trials. In a pharmacogenetic clinical trial there may be an analysis of the effect of a variance or variances on Phase I safety or surrogate

efficacy parameters. At the same time, evaluation of pharmacokinetic parameters (e.g., adsorption, distribution, metabolism, and excretion) may be a secondary objective; again, in a pharmacogenetic clinical study there may be an analysis of the effect of sequence variation in genes that affect absorption, distribution, metabolism and excretion of the candidate compound on pharmacokinetic parameters such as peak blood levels, half life or tissue distribution of the compound. As clinical development stages progress, trial objectives focus on the appropriate dose and method of administration required to elicit a clinically relevant therapeutic response. In a pharmacogenetic clinical trial, there may be a comparison of the effectiveness of several doses of a compound in patients with different genotypes, in order to identify interactions between genotype and optimal dose. For this purpose the doses selected for late stage clinical testing may be greater, equal or less than those chosen based upon preclinical safety and efficacy determinations. Data on the function of different alleles of genes affecting pharmacokinetic parameters could provide the basis for selecting an optimal dose or range or doses of a compound or biological. Genes involved in drug metabolism may be particularly useful to study in relation to understanding interpatient variation in optimal dose. Genes involved in drug metabolism include the cytochrome P450s, especially 2D6, 3A4, 2C9, 2E1, 2A6 and 1A1; the glucuronyltransferases; the acetyltransferases; the methyltransferases; the sulfotransferases; the glutathione system; the flavine monooxygenases and other enzymes known in the art.

An additional objective in the latter stages of clinical development is demonstration of the effect of the therapeutic intervention on a broad population. In phase III trials, the number of individuals enrolled is dictated by a power analysis. The number of patients required for a given pharmacogenetic clinical trial will be determined by prior knowledge of variance or haplotype frequency in the study population, likely response rate in the treated population, expected magnitude of pharmacogenetic effect (for example, the ratio of response rates between a genetic subgroup and the unfractionated population, or between two different genetic subgroups); nature of the genetic effect, if known (e.g. dominant effect, codominant effect, recessive effect); and number of genetic hypotheses to be evaluated (including number of genes and/or variances to be studied, number of gene or variance interactions to be studied). Other considerations will likely arise in the design of specific trials.

Clinical trials should be designed to blind both human subjects and study coordinators from biasing that may otherwise occur during the testing of a candidate therapeutic invention. Often the candidate therapeutic intervention is compared to best medical treatment, or a placebo (a compound, agent, device, or procedure that

appears identical to the candidate therapeutic intervention but is therapeutically inert). The combination of a placebo group and blind controls for potentially confounding factors such as prejudice on the part of study participants or investigators, insures that real, rather than perceived or expected, effects of the candidate therapeutic intervention are measured in the trials. Ideally blinding extends not only to trial subjects and investigators but also to data review committees, ancillary personnel, statisticians, and clinical trial monitors.

In pharmacogenetic clinical studies, a placebo arm or best medical control group may be required in order to ascertain the effect of the allelic variance or variances on the efficacy or toxicology of the candidate therapeutic intervention as well as placebo or best medical therapy. It will be important to assure that the composition of the control and test populations are matched, to the degree possible, with respect to genetic background and allele frequencies. This is particularly true if the variances being investigated may have an effect on disease manifestations (in addition to a hypothesized effect on response to treatment). It is likely that standard clinical trial procedures such as insuring that treatment and control groups are balanced for race, sex and age composition and other non-genetic factors relevant to disease will be sufficient to assure that genetic background is controlled, however a preferred practice is to explicitly test for genetic stratification between test and control groups. Methods for minimizing the possibility of spurious results attributable to genetic stratification between two comparison groups include the use of surrogate markers of geographic, racial and/or ethnic background, such as have been described by Rannala and coworkers. (See, for example: Rannala B, and JL Mountain. 1997 Detecting immigration by using multilocus genotypes. *Proc Natl Acad Sci USA* Aug 19;94(17):9197-201.) One procedure would be to assure that surrogate markers of genetic background (such as those described by Rannala and Mountain) occur at comparable frequency in two comparison groups.

Open label trials are unblinded; in single blind trials patients are kept unaware of treatment assignments; in double blind trials both patients and investigators are unaware of the treatment groups; a combination of these procedures may be instituted during the trial period. Pharmacogenetic clinical trial design may include one or a combination of open label, single blind, or double blind clinical trial designs. Reduction of biases attributable to the knowledge of either the type of treatment or the genotype of the normal subjects or patients is an important aspect of study design. So, for example, even in a study that is single blind with respect to treatment, it should be possible to keep both patients and caregivers blinded to genotype during the study.

In designing a clinical trial it is important to include termination endpoints

such as adverse clinical events, inadequate study participation either in the form of lack of adherence to the clinical protocol or loss to follow up, (e.g. such that adequate power is no longer assured), lack of adherence on the part of trial investigators to the trial protocol, or lack of efficacy or positive response within the test group. In a pharmacogenetic clinical trial these considerations obtain not only in the entire treatment group, but also in the genetically defined subgroups. That is, if a dangerous toxic effect manifests itself predominantly or exclusively in a genetically defined subpopulation of the total treatment population it may be deemed inappropriate to continue treating that genetically defined subgroup. Such decisions are typically made by a data safety monitoring committee, a group of experts not including the investigators, and generally not blinded to the analysis, who review the data from an ongoing trial on a regular basis.

It is important to note that medicine is a conservative field, and clinicians are unlikely to change their behavior on the basis of a single clinical trial. Thus it is likely that, in most instances, two or more clinical trials will be required to convince physicians that they should change their prescribing habits in view of genetic information. Large scale trials represent one approach to providing increased data supporting the utility of a genetic stratification. In such trials the stringent clinical and laboratory data collection characteristic of traditional trials is often relaxed in exchange for a larger patient population. Important goals in large scale pharmacogenetic trials will include establishing whether a pharmacogenetic effect is detectable in all segments of a population. For example, in the North American population one might seek to demonstrate a pharmacogenetic effect in people of African, Asian, European and Hispanic (i.e. Mexican and Puerto Rican) origin, as well as in native American people. (It generally will not be practical to segment patients by geographical origin in a standard clinical trial, due to loss of power.) Another goal of a large scale clinical trial may be to measure more precisely, and with greater confidence, the magnitude of a pharmacogenetic effect first identified in a smaller trial. Yet another undertaking in a large scale clinical trial may be to examine the interaction of an established pharmacogenetic variable (e.g. a variance in gene A, shown to affect treatment response in a smaller trial) with other genetic variances (either in gene A or in other candidate genes). A large scale trial provides the statistical power necessary to test such interactions.

In designing all of the above stages of clinical testing investigators must be attentive to the statistical problems raised by testing multiple different hypotheses, including multiple genetic hypotheses, in subsets of patients. Bonferroni's correction or other suitable statistical methods for taking account of multiple hypothesis testing will need to be judiciously applied. However, in the early stages

of clinical testing, when the main goal is to reduce the large number of potential hypotheses that could be tested to a few that will be tested, based on limited data, it may be impractical to rigidly apply the multiple testing correction.

5 B. Phase I Clinical Trials

1. Introduction

Phase I of clinical development is generally focused on safety, although drug companies are increasingly obtaining information on pharmacokinetics and surrogate pharmacodynamic markers in early trials. Phase I studies are typically performed with a small number (< 60) of normal, healthy volunteers usually at single institutions. The primary endpoints in these studies usually relate to pharmacokinetic parameters (i.e. adsorption, distribution, metabolism and bioavailability), and dose-related side effects. In a Phase I pharmacogenetic clinical trial; stratification based upon allelic variance or variances of a candidate gene or genes related to pharmacokinetic parameters may allow early assessment of potential genetic interactions with treatment.

Phase I studies of some diseases (e.g. cancer or other medically intractable diseases for which no effective medical alternative exists) may include patients who satisfy specified inclusion criteria. These safety/limited-efficacy studies can be conducted at multiple institutions to ensure rapid enrollment of patients. In a pharmacogenetic Phase I study that includes patients, or a mixture of patients and normals, the status of a variance or variances suspected to affect the efficacy of the candidate therapeutic intervention may be used as part of the inclusion criteria. Alternatively, analysis of variances or haplotypes in patients with different treatment responses may be among the the endpoints. It is not unusual for such a Phase I study design to include a double-blind, balanced, random-order, crossover sequence (separated by washout periods), with multiple doses on separate occasions and both pharmacokinetic and pharmacodynamic endpoints.

30 2. Phase I trials with subjects drawn from large populations and/or from related volunteer subjects: the Pharmacogenetic Phase I Unit concept

In general it is useful to be able to assess the contribution of genetic variation to treatment response at the earliest possible stage of clinical development. Such an assessment, if accurate, will allow efficient prioritization of candidate compounds for subsequent detailed pharmacogenetic studies; only those treatments where there is early evidence of a significant interaction of genetic variation with treatment response would be advanced to pharmacogenetic studies in later stages of development. In this invention we describe methods for achieving early insight – in

Phase I - into the contribution of genetic variation to variation in surrogate treatment response variables. It occurred to the inventors that this can be accomplished by bringing the power of genetic linkage analysis and outlier analysis to Phase I testing via the recruitment of a very large Phase I population including a large number of individuals who have consented in advance to genetic studies (occasionally referred to hereinafter as a Pharmacogenetic Phase I Unit). In one embodiment of a Pharmacogenetic Phase I Unit many of the subjects are related to each other by blood. (Currently Phase I trials are performed in unrelated individuals, and there is no consideration of genetic recruitment criteria, or of genetic analysis of surrogate markers.) There are several novel ways in which a large population, or a population comprised at least in part of related individuals, could be useful in early clinical trials. Some of the most attractive applications depend on the availability of surrogate markers for pharmacodynamic drug action which can be used early in clinical development, preferably in normal subjects in Phase I. Such surrogate markers are increasingly used in Phase I, as drug development companies seek to make early yes/no decisions about compounds.

Recruitment of a population optimized for clinical genetic investigation may entail utilization of methods in statistical genetics to select the size and composition of the population. For example powerful methods for detecting and mapping quantitative trait loci in sibpairs have been developed. These methods can provide some estimate of the statistical power derived from a given number of groups of closely related individuals. Ideally subjects in the pharmacogenetic Phase I unit are of known ethnic/racial/geographic background and willing to participate in Phase I studies, for pay, over a period of years. The population is preferably selected to achieve a specified degree of statistical power for genetic association studies, or is selected in order to be able to reliably identify a certain number of individuals with rare genotypes, as discussed below. Family participation could be encouraged by appropriate incentive compensation. For example, individual subjects might be paid \$200 for participation in a study; two sibs participating in the same study might each be paid \$300; if they could encourage another sib (or cousin) to participate the three related individuals might each be paid \$350, and so forth. This type of compensation would encourage subjects to recruit their relatives to participate in Phase I studies. (It would also increase the cost of studies, however the type of data that can be obtained can not be duplicated with conventional approaches.) The optimal location to establish such a Phase I unit is a city with a stable population, many large families, and a positive attitude about gene technology. The Pharmacogenetic Phase I Unit population can then be used to test for the existence of genetic variation in response to any drug as a first step in deciding whether to

proceed with extensive pharmacogenetic studies in later stages of clinical development. Specific uses of a large Phase I unit in which some or all subjects are related include:

- a. It should be possible, for virtually any compound, to assess the magnitude of the genetic contribution to variation in drug response (if any) by comparing variation in drug response traits among related vs. non-related individuals. The rationale is as follows: if a surrogate drug response trait (i.e. a surrogate marker of pharmacodynamic effect that can be measured in normal subjects) is under strong genetic control then related individuals, who share 25% (cousins) or 50% (sibs) of their alleles, should have less divergent responses (less intragroup variance) than unrelated individuals, who share a much smaller fraction of alleles. That is, individuals who share alleles at the genes that affect drug response should be more similar to each other (i.e. have a narrower distribution of responses, whether measured by variance, standard deviation or other means) than individuals who, on average, share very few alleles. By using statistical methods known in the art the degree of variation in a set of data from related individuals (each individual would only be compared with his/her relatives, but such comparisons would be performed within each group of relatives and a summary statistic developed) could be compared to the degree of variation in a set of unrelated individuals (the same subjects could be used, but the second comparison would be across related groups). Account would be taken of the degree of similarity expected between related individuals, based on the fraction of the genome they shared by descent. Thus the extent of variation in the surrogate response marker between identical twins should be less than between sibs, which should be less than between first cousins, which should be less than that between second cousins, and so forth, *if* there is a genetic component to the variation. It is well known from twin studies (in which, for example, variation between identical twins is compared to variation between fraternal twins) that pharmacokinetic variables (e.g. compound half life, peak concentration) are frequently over 90% heritable; the type of study proposed here (comparison of variation within groups of sibs and cousins to variation between unrelated subjects) would also show this genetic effect, without requiring the recruitment of monozygotic twins. For a summary of pharmacokinetic studies in twins see: Propping, Paul (1978) *Pharmacogenetics. Rev. Physiol. Biochem. Pharmacol.* 83: 123-173.

- It may be that the pattern of drug responses that distinguishes related individuals from non-related individuals is more complex than, for example, variance or standard deviation. For example, there may be two discrete phenotypes characteristic of intrafamilial variation (a bimodal distribution) that are not a feature

of variation between unrelated individuals (where, for example, variation might be more nearly continuous). Such a pattern could be attributable to Mendelian inheritance operating on a restricted set of alleles in a family (or families) with, for example, AA homozygotes giving one phenotype and AB heterozygotes and BB homozygotes giving a second phenotype, all in the context of a relatively homogeneous genetic background. In contrast, variation among non-related subjects would be less discrete due to a greater degree of variation in genetic background and the presence of additional alleles C, D and E at the candidate locus. Statistical measures of the significance of such differences in distribution, including nonparametric methods such as chi square and contingency tables, are known in the art.

The methods described herein for measuring whether pharmacodynamic traits are under genetic control, using surrogate markers of drug efficacy in phase I studies which include groups of related individuals, will be useful in obtaining an early assessment of the extent of genetically determined variation in drug response for a given therapeutic compound. Such information provides an informed basis for either stopping development at the earliest possible stage or, preferably, continuing with development but with a plan for identifying and controlling for genetic variation so as to allow rapid progression through the regulatory approval process.

For example, it is well known that Alzheimers trials are long and expensive, and most drugs are only effective in a fraction of patients. Using surrogate measures of response in normals drawn from a population of related individuals would help to assess the contribution of genetic variation to variation in treatment response. For an acetylcholinesterase inhibitor, relevant surrogate pharmacodynamic measures could include testing erythrocyte membrane acetylcholinesterase levels in drug treated normal subjects, or performing psychometric tests that are affected by treatment (and ideally that correlate with clinical efficacy) and measuring the effect of treatment. As another example, antidepressant drugs can produce a variety of effects on mood in normal subjects – or no effect at all. Careful monitoring and measurement of such responses in related vs. unrelated normal subjects, and statistical comparison of the degree of variation in each group, could provide an early readout on whether there is a genetic component to drug response (and hence clinical efficacy). The observation of similar effects in family members, and comparatively dissimilar effects in unrelated subjects would provide compelling evidence of a pharmacogenetic effect and justify the substantial expenditure necessary for a full pharmacogenetic drug development program. Conversely, the absence of any significant family influence on drug response would provide an early termination point for pharmacogenetic studies. *Note that the proposed studies do*

not require any knowledge of candidate genes, nor is DNA collection or genotyping required – simply a reliable surrogate pharmacodynamic assay and small groups of related normal individuals. Refined statistical methods should permit the magnitude of the pharmacogenetic effect to be measured, which could be a further criteria for deciding whether to proceed with pharmacogenetic analysis. The greater the differential in magnitude or pattern of variance between the related and the unrelated subjects, the greater the extent of genetic control of the trait.

Not all drug response traits are under the predominant control of one locus. Many such traits are under the control of multiple genes, and may be referred to as quantitative trait loci. It is then desirable to identify the major loci contributing to variation in the drug response trait. This can be done for example, to map quantitative trait loci in a population of drug treated related normals. Either a candidate gene approach or a genome wide scanning approach can be used. (For review of some relevant methods see: Hsu L, Aragaki C, Quiaoit F. (1999) A genome-wide scan for a simulated data set using two newly developed methods. *Genet Epidemiol* 17 Suppl 1:S621-6; Zhao LP, Aragaki C, Hsu L, Quiaoit F. (1998) Mapping of complex traits by single-nucleotide polymorphisms. *Am J Hum Genet* 63(1):225-40; Stoesz MR, Cohen JC, Mooser V, et al. (1997) Extension of the Haseman-Elston method to multiple alleles and multiple loci: theory and practice for candidate genes. *Ann Hum Genet* 61 (Pt 3):263-74.) However, this method would require at least 100 patients (preferably 200, and still more preferably >300) to have adequate statistical power, and each patient would have to be genotyped at a few polymorphic loci (candidate gene approach) or hundreds of polymorphic loci (genome scanning approach).

b. With a large Phase I population of normal subjects that need not be related (a second type of Pharmacogenetic Phase I Unit) it is possible to efficiently identify and recruit for any Phase I trial a set of individuals comprising virtually any combination of genotypes present in a population (for example, all common genotypes, or a group of genotypes expected to represent outliers for a drug response trait of interest). This method preferably entails obtaining blood or other tissue (e.g. buccal smear) in advance from a large number of the subjects in the Phase I unit. Ideally consent for genotyping would be obtained at the same time. It would be most efficient if blanket consent for genotyping any polymorphic site or sites could be obtained. Second best would be consent for testing any site relevant to any customer project (not specific at the time of initial consent). Third best would be consent to genotype polymorphic sites relevant to specific disease areas. Another, less desirable, solution would be to obtain consent for genotyping on a project by

project basis (for example by mailing out reply cards), after the specific polymorphic sites to be genotyped are known.

One useful way to screen for pharmacogenetic effects in Phase I is to recruit homozygotes for a variance or variances of interest in one or more candidate genes.

5 For example, consider a compound for which there are two genes that are strong candidates for influencing response to treatment. Gene X has alleles A and A', while gene Y has alleles B and B'. If these genes do in fact contribute significantly to response then one would expect that, regardless of the mode of inheritance (recessive, codominant, dominant, polygenic) homozygotes would exhibit the most

10 extreme responses. One would also expect epistatic interactions, if any, to be most extreme in double homozygotes. Thus one would ideally perform a surrogate drug response test in Phase I volunteers doubly homozygous at both X and Y. That is, test AA/BB, A'A'/BB, AA/B'B' and A'A'/B'B' subjects. If the allele frequencies for A and A' are .15 and .85, and for B and B' .2 and .8 then the frequency of AA

15 homozygotes is expected to be 2.25% and BB homozygotes 4%. In the absence of any linkage between the genes, the frequency of AA/BB double homozygotes is expected to be $0.0225 \times 0.04 = 0.0009$ or .09%, or about 1 subject in 1000. Ideally at least 5 subjects of each genotype are recruited for the Phase I study, and preferably at least 10 subject. Thus, even for variances of moderately low allele

20 frequency (15%, 20%), the identification of potential outliers (i.e. homozygotes) for the candidate genes of interest will require a large population. Preferably the Phase I unit has enrolled at least 1,000 normal individuals, more preferably 2,000, still more preferably 5,000 and most preferably 10,000 or more. In another application of the large, genotyped Phase I population it may be useful to identify individuals

25 with rare variances in candidate genes (either homozygous or heterozygous), in order to determine whether those variances are predisposing to extreme pharmacological responses to the compound. For example, variances occurring at 5% allele frequency are expected to occur in homozygous form in 0.25% of the population (0.05×0.05), and therefore may rarely, if ever, be encountered in early

30 clinical development. Yet it may be serious adverse effects occurring in just such a small group that create problems in later stages of drug development. In yet another application of the large genotyped Phase I population, subjects may be selected to represent the known common variances in one or more genes that are candidates for influencing the response to treatment. By insuring that all common genotypes are

35 represented in a Phase I trial the likelihood of misleading results due to genetic stratification (resulting in discrepancy with results of later, larger trials can be reduced.

It would be useful to prospectively genotype the large Phase I population for variances that are commonly the source of interpatient variation in drug response, since demand for genotyped groups of such patients can be anticipated from pharmaceutical companies and contract research organizations (CROs). For example, genotyping might initially focus on common pharmacological targets such as estrogen receptors, adrenergic receptors, or serotonin receptors. The pre-genotyped Phase I population could be part of a package of services (along with genotyping assay development capability, high throughput genotyping capacity and software and expertise in statistical genetics) designed to accelerate pharmacogenetic Phase I studies. Eventually, as the databank of genotypes built up, individuals with virtually any genotype or combination of genotypes could be called in for precisely designed physiological or toxicological studies designed to test for pharmacogenetic effects.

One of the most useful aspects of the Pharmacogenetic Phase I Unit is that subjects with rare genotypes can be pharmacologically assessed in a small study. This addresses a serious limitation of conventional clinical trials with respect to the investigation of polygenic traits or the effect of rare alleles. Unfortunately even Phase III studies, as currently performed, are often barely powered to address simple one variance hypotheses about efficacy or toxicity. The problem, of course, is that each time a new genetic variable is introduced the comparison groups are cut in halves or thirds (or even smaller groups if there are multiple haplotypes at each gene). It is therefore a challenging problem to test the interaction of several genes in determining drug response. Yet the character of drug response data in populations – there is often a continuous distribution of responses among different individuals – suggests that drug responses may often be mediated by several genes. (On the other hand, there are an increasing number of well documented single gene, or even single variance, pharmacogenetic effects in the literature, showing that it is possible to detect the effect of a single variance.) One approach to identifying pharmacogenetic effects is to focus on finding the single gene variances that have the largest effects. This approach can be undertaken within the scale of current clinical trials. However, in order to develop a test which predicts a large fraction of the quantitative variation in a drug response trait it may be desirable to test the effect of multiple genes, including the interaction of variances at different genes, which may be non-additive (referred to as epistasis). The Pharmacogenetic Phase I Unit provides a way to efficiently test for gene interactions or multigene effects by, for example, allowing easy identification of individuals who, on account of being homozygous at several loci of interest, should be outliers for the drug response phenotypes of interest if there is a gene x gene interaction. Testing drug response in a small number of such

individuals will provide a quick read on gene interaction. Obtaining genetic data on the pharmacodynamic action of a compound in Phase I should also provide a crude measure of allele effects – which variances or haplotypes increase pharmacological responses and which decrease them. This information is of great value in designing subsequent trials, as it constrains the number of hypotheses to be tested, thereby enabling powerful statistical designs. This is because when the effect of variances on drug response measures is unknown one is forced to statistically test all the possible effects of each allele (e.g. two tailed tests). As the number of genetically defined groups increases (e.g. as a result of multiple variances or haplotypes) there is a loss of statistical power due to multiple testing correction. On the other hand, if the relative phenotypic effect of each allele at a locus is known (or can be hypothesized) from Phase I data then each individual in a subsequent clinical trial contributes useful information – there is a specific prediction of response based on that individual's combination of genotypes or haplotypes, and testing the fit of the actual data to those predictions provides for powerful statistical designs. (It is also possible to measure allele effects biochemically, of course, to establish which alleles have positive and which negative effects, but at considerable cost.)

It is important to note that Phase I trials can provide useful information at almost any stage of clinical development. It is not unusual, for example, for a product in Phase II or even Phase III testing to be remanded to Phase I in order to clarify some aspect of toxicology or physiology. In this context a Pharmacogenetic Phase I Unit would be extremely useful to a drug development company. Phase I studies in defined genetic subgroups drawn from a large genotyped population, or in groups of related individuals, would be the most economical and efficient way to clarify the existence of pharmacogenetic effects, if any, paving the way for future rational development of the product.

C. Phase II Clinical Trials

Phase II studies generally include a limited number of patients (<100) who satisfy a set of predefined inclusion criteria and do not satisfy any predefined exclusion criteria of the trial protocol. Phase II studies can be conducted at single or multiple institutions. Inclusion/exclusion criteria may include historical, clinical and laboratory parameters for a disease, disorder, or condition; age; gender; reproductive status (i.e. pre- or postmenopausal); coexisting medical conditions; psychological, emotional or cognitive state, or other objective measures known to those skilled in the art. In a pharmacogenetic Phase II trial the inclusion/exclusion criteria may include one or more genotypes or haplotypes. Alternatively, genetic analysis may

be performed at the end of the trial. The primary goals in Phase II testing may include (i) identification of the optimal medical indication for the compound, (ii) definition of an optimal dose or range or doses, balancing safety and efficacy considerations (dose-finding studies), (iii) extended safety studies (complementing
5 Phase I safety studies), (iv) evaluation of efficacy in patients with the targeted disease or condition, either in comparison to placebo or to current best therapy. To some extent these goals may be achieved by performing multiple trials with different goals. Likewise, Phase II trials may be designed specifically to evaluate pharmacogenetic aspects of the drug candidate. Primary efficacy endpoints typically
10 focus on clinical benefit, while surrogate endpoints may measure treatment response variables such as clinical or laboratory parameters that track the progress or extent of disease, often at lesser time, cost or difficulty than the definitive endpoints. A good surrogate marker must be convincingly associated with the definitive outcome. Examples of surrogate endpoints include tumor size as a surrogate for
15 survival in cancer trials, and cholesterol levels as a surrogate for heart disease (e.g. myocardial infarction) in trials of lipid lowering cardiovascular drugs. Secondary endpoints supplement the primary endpoint and may be selected to help guide further clinical studies.

In a pharmacogenetic Phase II clinical trial, retrospective or prospective
20 design will include the stratification of patients based upon a variance or variances in a gene or genes suspected of affecting treatment response. The gene or genes may be involved in mediating pharmacodynamic or pharmacokinetic response to the candidate therapeutic intervention. The parameters evaluated in the genetically stratified trial population may include primary, secondary or surrogate endpoints.
25 Pharmacokinetic parameters - for example, dosage, absorption, toxicity, metabolism, or excretion - may also be evaluated in genetically stratified groups. Other parameters that may be assessed in parallel with genetic stratification include gender, race, ethnic or geographic origin (population history) or other demographic factors.

30 While it is optimal to initiate pharmacogenetic studies in phase I, as described above, it may be the case that pharmacogenetic studies are not considered until phase II, when problems relating either to efficacy or toxicity are first encountered. It is highly desirable to initiate pharmacogenetic studies no later than Phase II of a clinical development plan because (1) phase III studies tend to be large
35 and expensive - not an optimal setting in which to explore untested pharmacogenetic hypotheses; (2) phase III studies are typically designed to test one fairly narrow hypothesis regarding efficacy of one or a few dose levels in a specific disease or condition. Phase II studies are often numerous, and are intended to

provide a broad picture of the pharmacology of the candidate compound. This is a good setting for initial pharmacogenetic studies. Several pharmacogenetic hypotheses may be tested in phase II, with the goal of eliminating all but one or two.

5 D. Phase III Clinical Trials

Phase III studies are generally designed to measure efficacy of a new treatment in comparison to placebo or to an established treatment method. Phase II studies are often performed at multiple sites. The design of this type of trial includes power analysis to ensure the sufficient data will be gathered to demonstrate the anticipated effect, making assumptions about response rate based on earlier trials. As a result Phase III trials frequently include large numbers of patients (up to 5,000). Primary endpoints in Phase III studies may include reduction or arrest of disease progression, improvement of symptoms, increased longevity or increased disease-free longevity, or other clinical measures known in the art. In a pharmacogenetic Phase III clinical study, the endpoints may include determination of efficacy or toxicity in genetically defined subgroups. Preferably the genetic analysis of outcomes will be confined to an assessment of the impact of a small number of variances or haplotypes at a small number of genes, said variances having already been statistically associated with outcomes in earlier trials. Most preferably variances at only one or two genes will be assessed.

After successful completion of one or more Phase III studies, the data and information from all trials conducted to test a new treatment method are compiled into a New Drug Application (NDA) and submitted for review by the US FDA, which has authority to grant marketing approval in the US and its territories. The NDA includes the raw (unanalyzed) clinical data, i.e. the patient by patient measurements of primary and secondary endpoints, a statistical analysis of all of the included data, a document describing in detail any observed side effects, tabulation of all patients who dropped-out of trials and detailed reasons for their termination, and any other available data pertaining to ongoing *in vitro* or *in vivo* studies since the submission of the investigational new drug (IND) application. If pharmacoeconomic objectives are a part of the clinical trial design then data supporting cost or economic analyses are included in the NDA. In a pharmacogenetic clinical study, the pharmacoeconomic analyses may include genetically stratified assessment of the candidate therapeutic intervention in a cost benefit analysis, cost of illness study, cost minimization study, or cost utility analysis. The analysis may also be simultaneously stratified by standard criteria such as race/ethnicity/geographic origin, sex, age or other criteria. Data from a genetically stratified analysis may be used to support an application for approval for

marketing of the candidate therapeutic intervention.

E. Phase IV Clinical Trials

Phase IV studies occur after a therapeutic intervention has been approved for marketing, and are typically conducted for surveillance of safety, particularly occurrence of rare side effects. The other principal reason for Phase IV studies is to produce information and relationships useful for marketing a drug. In this regard pharmacogenetic analysis may be very useful in Phase IV trials. Consider, for example, a drug that is the fourth or fifth member of a drug class (say statins, or thiazidinediones or fluoropyrimidines) to obtain marketing approval, and which does not differ significantly in clinical effects – efficacy or safety - from other members of the drug class. The first, second and third drugs in the class will likely have a dominant market position (based on their earlier introduction into the marketplace) that is difficult to overcome, particularly in the absence of differentiating clinical effects. However, it is possible that the new drug produces a superior clinical effect – for example, higher response rate, greater magnitude of response or fewer side effects - in a genetically defined subgroup. The genetic subgroup with superior response may constitute a larger fraction of the total patient population than the new drug would likely achieve otherwise. In this instance, there is a clear rationale for performing a Phase IV pharmacogenetic trial to identify a variance or variances that mark a patient population with superior clinical response. Subsequently a marketing campaign can be designed to alert patients, physicians, pharmacy managers, managed care organizations and other parties that, with the use of a rapid and inexpensive genetic test to identify eligible patients, the new drug is superior to other members of the class (including the market leading first, second and third drugs introduced). The high responder subgroup defined by a variance or variances may also exhibit a superior response to other drugs in the class (a class pharmacogenetic effect), or the superior efficacy in the genetic subgroup may be specific to the drug tested (a compound-specific pharmacogenetic effect).

In a Phase IV pharmacogenetic clinical trial, both retrospective and prospective analysis can be performed. In both cases, the key element is genetic stratification based on a variance or variances or haplotype. Phase IV trials will often have adequate sample size to test more than one pharmacogenetic hypothesis in a statistically sound way.

F. Unconventional Clinical Development

Although the above listed phases of clinical development are well-established, there are cases where strict Phase I, II, III development does not occur,

for example, in the clinical development of candidate therapeutic interventions for debilitating or life threatening diseases, or for diseases where there is presently no available treatment. Some of the mechanisms established by the FDA for such studies include Treatment INDs, Fast-Track or Accelerated reviews, and Orphan Drug Status. In a clinical development program for a candidate therapeutic of this type there is a useful role for pharmacogenetic analysis, in that the candidate therapeutic may not produce a sufficient benefit in all patients to justify FDA approval, however analysis of outcome in genetic subgroups may lead to identification of a variance or variances that predict a response rate sufficient for FDA approval.

As used herein, "supplemental applications" are those in which a candidate therapeutic intervention is tested in a human clinical trial in order to gain an expanded label indication, expanding recommended use to new medical indications. In these applications, previous clinical studies of the therapeutic intervention, i.e. preclinical safety and Phase I human safety studies can be used to support the testing of the therapeutic intervention in a new indication. Pharmacogenetic analysis is also useful in the context of clinical trials to support supplemental applications. Since these are, by definition, focused on diseases not selected for initial development the overall efficacy may not be as great as for the leading indication(s). The identification of genetic subgroups with high response rates may enable the rapid approval of supplemental applications for expanded label indications. In such instances part of the label indication may be a description of the variance or variances that define the group with superior response.

As used herein, "outcomes" or "therapeutic outcomes" describe the results and value of healthcare intervention. Outcomes can be multi-dimensional, and may include improvement of symptoms; regression of a disease, disorder, or condition; prevention of a disease or symptom; cost savings or other measures.

Pharmacoeconomics is the analysis of a therapeutic intervention in a population of patients diagnosed with a disease, disorder, or condition that includes at least one of the following studies: cost of illness study (COI); cost benefit analysis (CBA), cost minimization analysis (CMA), or cost utility analysis (CUA), or an analysis comparing the relative costs of a therapeutic intervention with one or a group of other therapeutic interventions. In each of these studies, the cost of the treatment of a disease, disorder, or condition is compared among treatment groups. Costs have both direct (therapeutic interventions, hospitalization) and indirect (loss of productivity) components. Pharmacoeconomic factors may provide the motivation for pharmacogenetic analysis, particularly for expensive therapies that benefit only a fraction of patients. For example, interferon alpha is the only

treatment that can cure hepatitis C virus infection, however viral infection is completely and permanently eliminated in less than a quarter of patients. Nearly half of patients receive virtually no benefit from alfa interferon, but may suffer significant side effects. Treatment costs are ~\$10,000 per course. A
5 pharmacogenetic test that could predict responders would save much of the cost of treating patients not able to benefit from interferon alpha therapy, and could provide the rationale for treating a population in a cost efficient manner, where treatment would otherwise be unaffordable.

As used herein, "health-related quality of life" is a measure of the impact of a
10 disease, disorder, or condition on a patient's activities of daily living. An analysis of the health-related quality of life is often included in pharmacoeconomic studies.

As used herein, the term "stratification" refers to the partitioning of patients into groups on the basis of clinical or laboratory characteristics of the patient.

"Genetic stratification" refers to the partitioning of patients or normal subjects into
15 groups based on the presence or absence of a variance or variances in one or more genes. The stratification may be performed at the end of the trial, as part of the data analysis, or may come at the beginning of a trial, resulting in creation of distinct groups for statistical or other purposes.

20 *G. Power analysis in pharmacogenetic clinical trials*

The basic goal of power calculations in clinical trial design is to insure that trials have adequate patients and controls to fairly assess, with statistical significance, whether the candidate therapeutic intervention produces a clinically significant benefit.

25 Power calculations in clinical trials are related to the degree of variability of the drug response phenotypes measured and the treatment difference expected between comparison groups (e.g. between a treatment group and a control group). The smaller the variance within each group being compared, and the greater the difference in response between the two groups, the fewer patients are required to
30 produce convincing evidence of an effect of treatment. These two factors (variance and treatment difference) determine the degree of precision required to answer a specific clinical question.

The degree of precision may be expressed in terms of the maximal acceptable standard error of a measurement, the magnitude of variation in which the
35 95% confidence interval must be confined or the minimal magnitude of difference in a clinical or laboratory value that must be detectable (at a statistically significant level, and with a specified power for detection) in a comparison to be performed at

the end of the trial (hypothesis test). The minimal magnitude is generally set at the level that represents the minimal difference that would be considered of clinical importance.

In pharmacogenetic clinical trials there are two countervailing effects with respect to power. First, the comparison groups are reduced in size (compared to a conventional trial) due to genetic partitioning of both the treatment and control groups into two or more subgroups. However, it is reasonable to expect that variability for a trait is smaller within groups that are genetically homogeneous with respect to gene variances affecting the trait. If this is the case then power is increased as a function of the reduction in variability within (genetically defined) groups.

In general it is preferable to power a pharmacogenetic clinical trial to see an effect in the largest genetically defined subgroups. For example, for a variance with allele frequencies of 0.7 and 0.3 the common homozygote group will comprise 49% of all patients ($0.7 \times 0.7 \times 100$). It is most desirable to power the trial to observe an effect (either positive or a negative) in this group. If it is desirable to measure an effect of therapy in a small genetic group (for example, the 9% of patients homozygous for the rare allele) then genotyping should be considered as an enrollment criterion to insure a sufficient number of patients are enrolled to perform an adequately powered study.

Statistical methods for powering clinical trials are known in the art. See, for example: Shuster, J.J. (1990) Handbook of Sample Size Guidelines for Clinical Trials. CRC Press, Boca Raton, FL; Machin, D. and M.J. Campbell (1987) Statistical Tables for the Design of Clinical Trials. Blackwell, Oxford, UK; Donner, A. (1984) Approaches to Sample Size Estimation in the Design of Clinical Trials – A Review. *Statistics in Medicine* 3: 199-214.

H. Statistical analysis of clinical trial data

There are a variety of statistical methods for measuring the difference between two or more groups in a clinical trial. One skilled in the art will recognize that different methods are suited to different data sets. In general, there is a family of methods customarily used in clinical trials, and another family of methods customarily used in genetic epidemiological studies. Methods in quantitative and population genetics designed to measure the association between genotypes and phenotypes, and to map and measure the effect of quantitative trait loci are also relevant to the task of measuring the impact of a variance on response to a treatment. Methods from any of these disciplines may be suitable for performing statistical analysis of pharmacogenetic clinical trial data, as is known to those skilled in the art.

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